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NEW BLOOD FLUKES OF THE FAMILY SPIRORCHIDAE STUNKARD FROM INDIAN FRESH-WATER TORTOISES WITH DISCUSSION ON THE SYNONYMY OF CERTAIN GENERA AND THE RELATIONSHIPS OF THE FAMILIES OF BLOOD FLUKES.—PART II.

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INTRODUCTION

While we described two new species of the new genus *Coeuritrema* in a paper published in Vol. 2, No. 4 of this journal, we discussed the systematic position of that genus and relationships of the family Spirorchidae with the Schistosomatidae. It was shown in the course of discussion that the subfamily Hapalotreminae represents the primitive blood flukes, from which are evolved along one line the Schistosomidae and along the other close to the subfamily Spirorchinae, the blood flukes of fishes belonging to the families Aporocotylidae and Sanguinicolidae. In this paper are described four new species of blood flukes assigned to a new genus *Plasmiorchis*, which as will be seen from the description and subsequent discussion is closely related to the American genus *Spirorchis*. The resemblance between these two genera is so close that at first sight it appeared desirable to create two subgenera under the genus *Spirorchis*, one for the American species and the other for Indian forms described in this paper. But the closer examination revealed that the presence of a ventral sucker and forwardly directed loops of the intestinal caeca, one on each side of the oesophagus, are such constant features of the Indian species as to necessitate their inclusion in a separate genus, which we call *Plasmiorchis* on account of their habitat in the blood plasma of their hosts.

Odhner in 1911 while discussing the relationships of *Aporocotyle* with *Sanguinicola* pointed out that the H-shaped gut of the latter is derived by a great reduction in length of that of the former. He also mentioned that the

condition of the alimentary system in *Deontacylix* shows an intermediate condition between that of *Sanguinicola* and *Aporocotyle*. It seems obvious that we have got a descending series in the evolution of the alimentary system of the four genera of suckerless trematodes hitherto known, *Aporocotyle*—*Psettarium*—*Deontacylix*—*Sanguinicola*, all blood flukes of fishes. The presence of forwardly directed loops at the origin of caeca in *Plasmiorchis* gives us a clue to the origin of the anterior blindly ending lobes of the gut of the above-mentioned genera. It is, therefore, near the subfamily Spirorchinae particularly its genus *Plasmiorchis*, where we have to look for the ancestors of *Aporocotyle* and its descending series of genera *Psettarium*, *Deontacylix*, and *Sanguinicola*.

In view of the recent addition of a number of genera, the classification of the family Spirorchidae as given by Stunkard in 1923 has been revised. The genera *Henotosoma* Stunkard and *Haematotrema* Stunkard are held identical with the genus *Spirorchis* and are merged into it. The genus *Tremarhynchus* Thapar is held synonymous with *Coeuritrema*, the latter name being accepted on the basis of priority. *Tremarhynchus indicus* is consequently assigned as the third species to that genus under the name of *Coeuritrema indicus* (Thapar, 1933).

***Plasmiorchis orientalis* nov. gen., nov. spec.**

(Figs. 1, 4, 8, 9)

Three mature specimens were obtained in August 1931 and two in September 1932 from the ventricle of the heart of two water tortoises of the species *Kachuga dhongoka* at Allahabad. The blood flukes came out as soon as the ventricle was opened in salt solution and appeared inactive indicating no movements. The body is elongated fusiform or spindle-shaped, slightly tapering towards ends like that of the genus *Spirorchis*; it is thin specially at edges, flattened and transparent with the anterior end a little more pointed than the posterior. The size is very small, measuring 2.26\*—3 in length and 0.4–0.6 in greatest breadth, which lies about the middle of body length. The breadth measures 0.38–0.5 in the region of the intestinal bifurcation, 0.27–0.56 in that of the ventral sucker and 0.34–0.56 in that of the ovary, varying within the narrow limits of 0.33–0.4 or 0.5–0.6 between the intestinal bifurcation and the ovary. The body wall is covered with very thin cuticle and is devoid of papillae, but it is covered with fine needle-like spines, which hardly project outside it. The musculature of the body is poorly developed.

The oral sucker is oval, longer than broad and protrusible, measuring 0.102–0.108 in length and 0.066 in maximum breadth. It protrudes ordinarily

\* All measurements are given in millimeters.

only a little in front of the anterior end; in none of my specimens it is half or entirely protruded. The ventral sucker is well developed, protrusible and rounded, measuring 0'128–0'144 in diameter: in one specimen of 2'5 length it measures 0'081 in length and 0'087 in breadth, while the oral sucker in this specimen measures 0'102 in length and 0'066 in breadth. The ventral sucker lies 0'192–0'272 behind the intestinal bifurcation and 0'73–0'75 behind the anterior end, *i.e.*, at about one third body length from the anterior end: in one specimen, however, it lies between one third and one fourth body length from the latter. The pharynx is absent. The oesophagus 0'4 in length and 0'066–0'069 in greatest breadth is long and sinuous with two to four bends, extending up to 0'48 length of the body from the anterior end, *i.e.* about anterior one sixth body length. It is surrounded by deeply staining salivary gland cells, which are found in large numbers around its posterior part. Its inner wall appears plicated on account of the continuous discharge of salivary secretion through it into the lumen: the plications are more pronounced in the terminal part immediately in front of the intestinal bifurcation. At the point where it bifurcates into the caeca, it gives off behind the origin of the latter a small median pocket, the oesophageal vesicle. The caeca do not pass laterally as soon as they arise as in the genus *Spirorchis*, but they turn abruptly forwards running one on each side of the oesophagus for nearly one third or one half of its length and then bend downwards forming characteristic U-shaped loops as they continue their course to the hinder end. The presence of these forwardly directed loops is a characteristic feature of all the species belonging to the new genus. The caeca then run almost straight but for a short outward bend displayed by the left caecum in the region of the genital pore, the genital loop and terminate near the hinder end, where they converge a little towards each other lying parallel for a short distance. They are simple without diverticula, having more or less uniform breadth of 0'075, and lie half way between the body wall and median line except near their ends.

The excretory pore is slightly dorsal at the posterior end. The excretory bladder has a very small stem bifurcating into two wider branches, which could be traced up to the blind ends of the caeca. The glandular vesicle, which Stunkard considers as the lymph vesicle in the genus *Spirorchis* is not seen in sexually mature specimens. In immature specimens, however, it is present at the hinder end in the median line behind the vitelline reservoir, having a curved S-shaped appearance.

Main parts of the nervous system are visible in entire mounts. The oesophageal commissure is fairly prominent. It lies 0'096 distance behind the oral sucker, *i.e.*, at about one fourth distance from it and is slightly swollen on each side to form an indistinct ganglionic mass from which the main lateral nerve arises. The lateral nerves run posteriorly close outside the caeca on the ventral surface of the body as in *Spirorchis*.



The reproductive organs resemble essentially those of the genus *Spirorchis*. The testes, 5-7 in number, lie in a linear series in the median plane usually in close contact with one another, almost filling the intercaecal region between the ventral sucker and ovary. They are irregularly lobed, elliptical or ovoid in shape, flattened and broader than long, measuring 0.14-0.24 in length and 0.208-0.24 in breadth. The anterior and posterior testes are larger than the middle ones. The anterior testis lies a little behind the ventral sucker, 0.03-0.12 distance behind it and 0.033-0.53 behind the intestinal bifurcation. The testicular area occupies a little less than half the length of the body. There is no vas deferens. The vesicula seminalis, filled with sperms, is pear-shaped with the widest anterior end pressed against the left part of the posterior margin of the hindmost testis, situated usually to the left side and slightly overlapped by the inner margin of the ovary, filling almost the entire space between it and the left caecum. In a few specimens, however, it lies almost entirely ventral to the ovary, commencing a little in front of the latter, from the posterior margin of the hindmost testis. It extends backwards as far as the posterior limit of the ovary or a little behind it to the left side near the left caecum, where it enters the cirrus sac, measuring 0.27 in length and 0.075-0.105 in maximum breadth in its widest anterior end. The cirrus sac is extremely small with poorly developed musculature, somewhat oval or pear-shaped with the narrow end opening at the genital pore, and situated beneath or close inside the left caecum, measuring 0.09-0.13 in length and 0.03-0.054 in maximum breadth at its end near the vesicula seminalis. It contains inside a small vesicula seminalis interna of rounded or oval outline and of 0.045 diameter, followed by a sharply constricted off ductus ejaculatorius. The latter is pear-shaped, measuring 0.09 in length and 0.03 in greatest breadth near its proximal end. The genital opening lies 0.28-0.29 distance in front of the hinder end, and a little, *i.e.*, 0.128 distance behind the ovary, to the left side beneath the left caecum, where the latter is slightly bent outwards to form the characteristic genital loop. The cirrus sac opens anteriorly to the metraterm at the genital pore. The prostate gland cells are absent.

The ovary is much lobed, and lies median or slightly to the right side with its outer wall close inside the right caecum, immediately behind the hindmost testis at a distance of 0.4-0.6 from the posterior end, *i.e.*, at about one fifth body length from it. It overlaps partly or entirely the basal part of the vesicula seminalis, measuring 0.24 by 0.176, 0.176 by 0.112 and 0.9 by 0.9 in size in the three specimens examined for the purpose. The oviduct originates from the middle of its posterior margin, and after running a short distance dorsally backwards joins the receptaculum seminis which is filled with sperms. The receptaculum seminis is of an oval or spherical shape, and lies to the right side close behind the ovary, measuring 0.045-0.06 in

length and 0.05—0.075 in maximum breadth. We follow Ejsmont in calling this vesicle, the receptaculum seminis instead of receptaculum seminis uterinum as Ward and Stunkard have called it. The oviduct leaves the latter at the hinder end, and receives the small Laurer's canal; it then continues its course slightly to the left side, a little behind the level of the genital opening to receive the yolk reservoir, where it sharply turns forwards to open into the broad oval ootype or uterus, which contains a single large ovum. The uterus lies transversely, closely in front of the union of the transverse vitelline ducts, behind the receptaculum seminis, and enters terminally into a small metraterm with thin muscular walls. The latter opens to the exterior at the genital opening. The ovum is large, non-operculated and oval without filaments or spines, measuring 0.102—0.12 in length and 0.042—0.057 in maximum breadth.

The vitellaria are voluminous, and occupy lateral areas surrounding the caeca, extending from the middle of the oesophagus; *i.e.*, from the forward limits of the anterior loops of the caeca to almost the posterior end of the body. The follicles of small size lie mainly outside the caeca forming a continuous linear mass, the extracaecal areas, but they enter also the intracaecal region both dorsally and ventrally to form intracaecal areas. At the posterior end they generally meet in the intracaecal region between the blind ends of the caeca. The transverse vitelline ducts arise slantingly at about the level of the genital opening, the right a little in front of the left one, and unite closely behind the uterus near the ventral wall to form the backwardly directed vitelline reservoir, the narrow anterior end of which bends forwards on the dorsal side to open into the oviduct, near its junction with the uterus.

Four immature specimens were obtained from the ventricle of the heart of two *Kachuga dhongoka* (2 from each) dissected in November 1931. The body measures 1.5—1.9 in length and 0.37—0.54 in maximum breadth, which lies in the region between the ventral sucker and the ovary. The shape is elongated and fusiform with bluntly pointed ends. The oral sucker is oval and partly protrusible, measuring 0.09 by 0.05, and 0.1 by 0.06 in size in the two specimens examined for the purpose. The ventral sucker, 0.08—0.09 in diameter lies at about one third body length from the anterior end and 0.16—0.17 behind the intestinal bifurcation. The oesophagus, oesophageal vesicle and intestinal caeca with their characteristic loops are similar to those in mature specimens. The testes, 5—7 in number, are fairly large in size, containing mature sperms. The anterior testis, which is the smallest lies 0.096—0.135 behind the ventral sucker. The vesicula seminalis and ovary though small are developed in only one of the four specimens. The cirrus sac is indistinguishable. The vitellaria are entirely absent. The receptaculum seminis, present in only one specimen which possesses a very small slightly lobed ovary, is filled with sperms. From the study of immature specimens it is clear that the male reproductive organs are developed first and that the sperms for fertilisation are received in the

receptaculum seminis before the ovary is fully developed and functional. The vitellaria are developed last

Host—*Kachuga dhongoka*

Habitat—Ventricle of heart.

Locality—Allahabad, India.

***Plasmiorchis pellucidus* sp. n.**

(Figs. 2, 5, 10)

Two mature and one immature specimens of this species were obtained from the ventricle of the heart of *Kachuga dhongoka* in November 1931. The body is very thin and transparent, measuring 3.3.26 in length and 0.64-0.68 in greatest breadth, which lies in the region from the ventral sucker to the ovary. The shape is fusiform with bluntly pointed ends as in the genus *Spirorchis*.

The oral sucker is oval, longer than broad, and protrusible, measuring 0.126-0.132 in length and 0.087-0.09 in greatest breadth. The ventral sucker is absent in mature specimens, but its position is indicated by a definite clear region devoid of musculature or body parenchyma. This region where the ventral sucker was present and has obviously dropped off leaving a clear empty space in the body, we prefer to call the ventral sucker area. In immature specimen, however, the ventral sucker is present. This condition shows clearly how the ventral sucker has been secondarily lost in the genus *Spirorchis*. The ventral sucker area lies at about the end of the anterior one third body length, and measures 0.24-0.32 in diameter. The pharynx is absent. The oesophagus is long and sinuous with four bends, measuring 0.64 in length. It is more or less of uniform breadth except anteriorly where it is slightly narrower. It is surrounded by salivary gland cells, which are more numerous and densely crowded around its posterior end, before it passes into the vesicle; its inner wall appears plicated on account of the salivary secretion, which passes through it into the lumen. The oesophageal vesicle is well developed, measuring 0.096 in length and 0.08 in breadth. The intestinal caeca arise at the junction of the vesicle with the oesophagus and soon turn abruptly forwards running parallel to the latter for about posterior half of its length till they bend downwards forming the characteristic loops on their way backwards, ending almost at the hinder end of the body. The caeca are simple without diverticula, much narrower than the oesophagus, and run almost straight till the genital pore where the left caecum is slightly bent outwards to form the inconspicuous genital loop, which is less marked than that in the previous species. They lie almost half way between the body wall and the median line, converging slightly inwards towards each other behind the genital opening. The excretory pore is slightly dorsal. The glandular vesicle



is not visible in mature specimens. The nervous system is similar to that of the previous species

The testes, 8—9 in number, lie in a linear series in the median line, close behind and in contact with one another, separated on either side by a considerable distance from the corresponding intestinal caecum. They are of varying shapes, but usually ovoid with entire margins, and broader than long, measuring 0.09—0.165 in length and 0.105—0.175 in maximum breadth; the third and fourth testes are usually the smallest. The first testis lies a little behind the ventral sucker area, 0.08 distance behind it; the testicular area occupies a little less than half the length of the body. The vas deferens is absent. The vesicula seminalis is filled with sperms, and lies as in the previous species immediately behind the hindmost testis with its anterior dilated part interposed between it and the ovary in the median line. It is pear-shaped with the narrow tubular part directed to the left side towards the genital opening, where it enters the small cirrus sac; anteriorly it is overlapped for the greater part of its length by the left half of the dorsally situated ovary. The cirrus sac, 0.18 in length and 0.045 in maximum breadth, is tubular, commencing a little behind the ovary, at about the level of the receptaculum seminis near the left caecum. It contains inside a small portion of the vesicula seminalis, constricted off from the larger distally situated ductus ejaculatorius filled with sperms. The genital opening lies to the left side, a little behind the receptaculum seminis, at a distance of 0.35 from the hinder end beneath the left caecum, where the latter slightly bends outwards to form the inconspicuous genital loop.

The ovary lies median or slightly to the right side with its outer wall close inside the right caecum, closely behind the hindmost testis, at a distance of 0.5—0.58 from the hinder end, *i.e.*, about one fifth body length from it. It is divided into four or five lobes, and has nearly equal long and broad diameters, measuring 0.17—0.2 by 0.17—0.2 in size. The oviduct originates from the middle of the hinder margin of the ovary, and runs for a short distance before it joins the oval or spherical receptaculum seminis filled with sperms. The receptaculum seminis, 0.072—0.8 × 0.09 in size, lies closely behind the ovary, to the right side near the right caecum, at 0.43 distance in front of the hinder end. The oviduct leaves the receptaculum seminis at the hinder end near its outer margin, and passes a little backwards to the median line behind the level of the genital opening to join the narrow anterior end of the vitelline reservoir; soon after it sharply turns on itself to continue forwards as the prominent uterus, distended with the single ovum contained in it. The uterus lies transversely as in the previous species, a little behind the receptaculum seminis to the left side, and terminates into the short narrow metraterm, which opens to the exterior at the genital opening. Laurer's canal is very small and inconspicuous. The shell gland cells are absent. The

ovum is large, non-operculated and oval without filaments, measuring 0.136 in length and 0.095 in maximum breadth.

The vitellaria are well developed, extending from about the middle of the oesophagus, *i.e.*, from the anterior limits of the anterior loops of the caeca to a little in front of the hinder end of the body. They are composed of follicles of fairly large size, restricted mostly to the extracaecal region and overlapping the caeca at places. The follicles hardly extend inside the caeca, except behind the vitelline reservoir and in the region of the anterior loops, where they tend to meet in the median line. The transverse vitelline ducts arise obliquely, the right one at about the level of the genital opening and the left one a little behind it, and unite just behind the uterus near the ventral body wall to form the vitelline reservoir, which ends blindly to the left side near the left caecum.

*Plasmiorchis pellucidus* is distinguished from *P. orientalis* in a number of important features. The ventral sucker is absent in mature specimens; its position, however, can be easily determined by the presence of a clear transparent region free from the parenchyma, which is called the ventral sucker area. The testes are larger in number and are entire or slightly lobed, whereas in *P. orientalis* they are much lobed and lie quite apart from one another, filling almost the entire breadth of the intracaecal region. The length of the oesophagus and the anterior loops of the caeca is greater in *P. pellucidus* than in the other species. The vitelline follicles are mostly restricted to the extracaecal areas and do not enter the intracaecal region outside the testes as in *P. orientalis*.

Host—*Kachuga dhongoka*.

Habitat—Ventricle of heart.

Locality—Allahabad, India

### ***Plasmiorchis hardellii* sp. n.**

(Figs 3, 6, 11, 12, 13)

These blood flukes were collected in November and December 1931 and August 1932 from the ventricle of the heart and aortic arches of the water tortoises *Hardella thurgi*, which are not commonly available at Allahabad. Out of the five tortoises examined three were found infected, with nine, one and five parasites each; of these we possess nine specimens in entire mounts and others in longitudinal and transverse sections. When freed in normal salt solution they move slowly with gliding movements. The body is thin, transparent, elongated and elliptical in shape with rounded anterior and posterior ends; the anterior end is generally broader and more rounded, but sometimes it is bluntly pointed on account of the partly protruding oral sucker. The size is larger than that of the other species, measuring in entire

TABLE SHOWING MEASUREMENTS OF *Plasmodium hardellii*.

No.	Length of body.	Breadth in the region of ventral sucker.	Greatest breadth.	Oral sucker.	Ventral sucker.	Length in front of ventral sucker.	Length behind ventral sucker.	Genital opening: distance in front of hinder end.	Ovary: distance in front of hinder end
1.	5.28	1.36	1.57	0.24 × 0.176	0.41 × 0.64	1.44	3.42	0.62	0.75
2.	5.15	1.23	1.39	0.22 × 0.176	0.38 × 0.57	1.44	3.33	0.6	0.72
3.	4.38	1.02	1.34	0.16 × 0.128	0.304 × 0.368	1.23	2.816	0.624	0.672
4.	4.53	1.104	1.06	0.24 × 0.192	0.37 × 0.48	1.12	3.04	0.45	0.528
5.	3.85	1.088	1.18	0.21 × 0.176	0.368 × 0.48	1.12	2.37	0.38	0.45



mounts 5.55—5.28 in length and 1.18—1.57 in maximum breadth, which lies in the region midway between the ventral sucker and the ovary, *i.e.*, about the middle of the body length. The body wall has a thin muscular layer, which is covered outside by a thin cuticle. It is devoid of papillae, but it is armed with fine needle-like spines, which hardly project outside it.

The oral sucker is longer than broad and slightly protrusible, measuring ordinarily 0.24 in length and 0.176 in maximum breadth; in one specimen it measured 0.16 by 0.13 and in another 0.21 by 0.19 in size. The ventral sucker is broader than long and about double the size of the oral sucker, measuring 0.3—0.4 in length and 0.36—0.64 in maximum breadth. It is muscular, having a well developed layer of radial muscles with an outer thin layer of longitudinal muscle fibres, and lies 0.29 distance behind the intestinal bifurcation, at about one third body length from the anterior end. Its exact position can be seen from the table in which the length of the body in front and behind it is given in specimens of different lengths. The pharynx is absent. The oesophagus is long and slightly undulating with two or three bends, measuring 0.67—0.96 in length, *i.e.*, one fifth to one sixth part of the body length. It gradually increases in breadth towards the hinder end, and is surrounded by deeply staining salivary gland cells, which are found in much larger numbers around its hinder part. Its inner wall is plicated as in the other species. The oesophageal vesicle is well developed. The intestinal caeca arise at the junction of the vesicle with the oesophagus, and soon turn forwards to form the characteristic loops as described in the other species, which run parallel to the oesophagus for about the posterior three fourth of its length. The caeca of a much narrower calibre than the oesophagus possess small irregular diverticula, and form well defined loops in the region of the genital opening, close behind the ovary, before they terminate near the hinder end of the body. The genital loop formed by the left caecum has a characteristic semicircular shape, and is much wider than the corresponding loop of the right caecum, which lies closely behind the ovary with the bend directed inwards. These loops are specially well developed in this species; their hinder border marks out the anterior limit of the region, in which the glandular sac lies.

The excretory opening lies dorsal a little in front of the hinder end. The excretory bladder has a very short median stem, which bifurcates into two long narrow ducts running one on each side outside the caeca throughout the body length. The glandular vesicle is a large convoluted tubular mass, occupying the entire intracaecal space behind the genital loops of the caeca, *i.e.*, behind the ovary and the genital opening, and measuring 0.54 in length and 0.51 in maximum breadth near its anterior end. The presence of a large convoluted glandular vesicle, which is closely pressed against the walls of the caeca near their blind ends is a distinctive feature of this species. Histologically the walls of this tubular mass consist of an epithelium of cells, which

have lost their outlines and in most cases their nuclei; a few nuclei are, however, present in a degenerate condition here and there in the epithelium, which is apparently converted into a secretion. The secretion also fills the lumen of the vesicle in adult specimens. There is no muscular layer outside the epithelium. The glandular tubular mass ends blindly in the body, and is not of the nature of a lymph receptacle. It appears to be an important gland, which secretes a fluid of a colloidal nature, to be possibly absorbed within the body for some unknown physiological needs of the animal.

The nervous system resembles closely that of the other species. The oesophageal commissure, conspicuous in entire mounts, lies close in front of the anterior limits of the anterior loops of the intestinal caeca, 0.021 distance behind the anterior end of the oesophagus and 0.41 behind the anterior end of the body.

The testes, 19–21 in number, lie in a linear series in the median line, 0.064–0.096 distance behind one another, separated on either side by a moderate distance from the corresponding intestinal caecum. They are much broader than long with a characteristic band-like irregular form, narrow antero-posteriorly, thicker in the middle, pointed or somewhat notched at their lateral ends, and produced into one or two very short-pointed outgrowths near the middle region. Four or five anterior and a few posterior testes have the smallest breadth, measuring 0.032–0.1 in length and 0.24–0.27 in greatest breadth. The largest testes, situated about the middle of the row measure 0.032 in length and 0.4–0.48 in maximum breadth. The foremost testis lies a little distance behind the ventral sucker (0.128 behind it) and a little behind one third body length from the anterior end; the hindmost testis lies a little in front of the ovary and at a distance of 0.88 from the posterior end of the body. The testicular area occupies a little less than half the length of the body. In immature specimens the testes are seen developing in irregular chink-like spaces, marked out in the central deeply staining body parenchyma behind the ventral sucker. The vas deferens is absent and the vesicula seminalis poorly developed. In none of my specimens the latter is visible on account of the absence of sperms in it; it is represented, however, by a small median space between the hindmost testis and the cirrus sac. The cirrus sac is large, and unlike that of the other species has thick muscular walls composed of an inner layer of longitudinal and outer layer of circular muscle fibres. It is situated obliquely with the basal end near the median line just in front of or in level with the anterior margin of the ovary, a little behind the hindmost testis, and the terminal end to the left side close inside the left caecum within the region enclosed by its genital loop, 0.16 distance from the left body margin, and 0.5–0.67 distance in front of the hinder end.

The ovary lies to the right side closely inside the right caecum, 0.112 behind the hindmost testis and one fifth to one seventh part of the body

length in front of the hinder end, 0.45–0.75 in front of the latter. It is irregularly lobed and small in size, measuring 0.075–0.08 in length and 0.09–0.2 in maximum breadth. The oviduct arises from its inner margin, and runs backwards for a short distance to become enlarged into the receptaculum seminis, which in most of the specimens is not filled with sperms. The receptaculum seminis is somewhat pear-shaped, measuring 0.072–0.075 in length and 0.03–0.033 in maximum breadth. It becomes narrowed at its hinder end to pass into the uterus, which runs transversely from the median line to the left side. The uterus, 0.108 in length and 0.021–0.024 in breadth, is thin walled lined with parenchymatous cells, and passes at its terminal end into the metraterm of 0.108 length, which is lined internally with a thin cuticular layer, and shows peculiar elevations and depressions of its modified epithelium devoid of nuclei. The metraterm, however, is devoid of a muscular layer except in the small terminal part, the muscular tube, which is covered by a strongly developed musculature, functioning as a sphincter; the latter has no epithelial layer inside, and runs vertically downwards to open at the genital opening. The shell gland cells are absent. A small inconspicuous Laurer's canal is present. The ovum is large, elongated oval in shape, and non-operculated without filaments, measuring 0.081 in length and 0.03 in greatest breadth. Only one ovum is contained at a time in the uterus or the metraterm.

The vitellaria are well developed in a few specimens in my collection. They are situated laterally as in the other species, commencing a little in front of the middle of the oesophagus and terminating at the blind ends of the caeca. The follicles are smaller than those of the other species and are mostly aggregated outside the caeca, extending slightly inwards in the lateral areas outside the testicular zone. The transverse vitelline ducts lie just in front of the convoluted glandular vesicle, the right immediately behind the ovary and the left a little further behind. The vitelline reservoir lies immediately behind the transverse ducts in the median line, ending blindly just in front of the glandular vesicle. A fairly large number of specimens possessed the testes, cirrus sac, ovary, uterus and metraterm, but they lacked the vitellaria, which were obviously not developed. In a few specimens the latter were found developing as a mass of nuclei situated here and there in the extracaecal and intracaecal areas. The vesicula seminalis and receptaculum seminis were free from sperms in these relatively immature worms.

*Plasmiorchis hardellii* is distinguished from the other species in the large size and shape of the body, characteristic irregular shape of the testes, enormous development of the convoluted glandular vesicle, large size of the cirrus sac, small size of the ovary, slight development of the vesicula seminalis and presence of large well marked genital loops of the caeca in the region of the genital opening. The cirrus sac has thick muscular walls, while its musculature in the other species is weakly developed as in the genus



*Spirorchis*. The vitellaria are composed of follicles of small size. The metraterm is characterised by the presence of a highly modified epithelium showing elevations and depressions. It is devoid of musculature except in the terminal part, which has a strongly developed musculature functioning as a sphincter. The intestinal caeca have indented margins or small diverticula throughout their course.

Habitat—ventricle of heart and aortic arches.

Host—*Hardella thurgi*.

Locality—Allahabad, India.

***Plasmiorchis obscurum* sp. n.**

(Fig. 7)

Three immature specimens of this species were obtained from the ventricle of the heart of two specimens of *Kachuga dhongka*, one from one host and two from the other. Body length 3.2, maximum breadth 0.64 about the middle of body length; breadth in the region of ventral sucker 0.5. Body thin, transparent, elongated and spindle-shaped with bluntly pointed ends, broader near the anterior than near the posterior end. Suckers well developed. Oral sucker oval, entirely protrusible and twice as long as broad, measuring  $0.176 \times 0.08$ . Ventral sucker a little in front of one third body length, circular in outline, 0.144 in diameter. Pharynx absent. Oesophagus long, slightly undulating, nearly straight in the extended condition, 0.56 in length and 0.128 in maximum breadth, surrounded by salivary gland cells. Oesophageal vesicle well developed, 0.096 in length and 0.128 in breadth, ending 0.144 distance in front of ventral sucker. Anterior loops of intestinal caeca one on each side of oesophagus as in the other species, 0.384 in length. Intestinal caeca approaching towards each other and slightly undulating near their hinder end, with small outgrowths given off mainly from the inner walls and ending a little in front of the hinder end. A small, tubular, nearly straight glandular vesicle present in the median line at the hinder end of the body. Excretory bladder with a very short median stem bifurcating just behind the glandular vesicle into two long narrow ducts extending throughout the body length outside the intestinal caeca.

Intracaecal region between oesophageal pouch and rudiment of ovary a deeply staining mass of cells with rudiments of testes lying in a series in the median line. Testes rudimentary and hence small in size, about nineteen in number, separated from one another by  $0.048-0.08$  distance; posterior testes situated nearer one another than anterior testis. Ovary small and rudimentary. Vitellaria not yet developed.

The specimens though sexually immature with poorly developed genital organs, show certain well marked features, in which they are distinguishable

from the other species, such as the shape of the body, intestinal caeca undulating near the hinder end and provided with small outgrowths and large number of testes. This species differs from *P. hardellii* in size, shape of body, presence of a small glandular vesicle and in the size of the suckers and oesophagus. It comes nearer *P. orientalis* in shape and size of the body, presence of two suckers, size of ventral sucker, length of oesophagus and small size of glandular vesicle, but it differs in the intestinal caeca, which are undulating near the hinder end and possess small outgrowths. Moreover, it has a much larger number of testes than *P. orientalis*.

Habitat—Ventricle of heart.

Host—*Kachuga dhongoka*.

Locality—Allahabad, India.

### • Diagnosis of the Genus **Plasmiorchis** N. G.

Spirorchinae: Hermaphrodite distome blood flukes; delicate musculature. Body thin, elongated, flattened, narrow and elliptical or spindle shaped as in *Spirorchis*; body wall covered with very thin cuticle having fine needle like spines hardly projecting outside it. Oral sucker oval and protrusible; ventral sucker present (absent only in adult *Plasmiorchis pellucidus*, though ventral sucker area present), situated at about one third body length from anterior end. Pharynx absent; oesophagus long, about one fifth to one sixth length of body, sinuous with two to four bends and surrounded by salivary gland cells, which are numerous near its hinder end; inner wall of oesophagus plicated; small median pocket the oesophageal vesicle present at the point of intestinal bifurcation a little in front of ventral sucker. Intestinal caeca do not pass laterally as they arise, but turn abruptly forwards for one third to three fourths length of oesophagus and then bend backwards to form forwardly directed U-shaped loops, one on each side, continuing their course backwards almost to posterior end of body and forming small or well marked loops, specially the left caecum, in the region of genital pore. Genital opening ventral to the left side, beneath or close inside left intestinal caecum, behind ovary and a little in front of hinder end. Testes in a large number, arranged in a linear series in the median plane in the intracaecal area, behind ventral sucker and in front of ovary, usually irregularly lobed and of varying shapes; testicular area occupies a little less than half the length of body; vas deferens absent; vesicula seminalis usually well developed, filled with sperms and pear-shaped with its widest anterior end in contact with the hindmost testis. Cirrus sac extremely small with weak musculature except in *P. hardellii*, in which it is fairly large with stout musculature (vesicula seminalis small in this species); vesicula seminalis interna and ductus ejaculatorious present. Ovary lobed, median or slightly to the right, immediately behind hindmost testis,

about one fifth body length in front of hinder end; receptaculum seminis oval or rounded immediately behind ovary; uterus very short distinguished by the presence of a single large ovum, just behind receptaculum seminis; metraterm very short. Laurer's canal present and shell gland cells absent. Transverse vitelline ducts slantingly situated, right a little in front of the left one, immediately behind uterus; yolk reservoir behind transverse ducts, ending blindly to the left side. Vitellaria voluminous and situated laterally, extending from about middle of oesophagus, *i.e.*, from anterior limits of forwardly directed loops of caeca to posterior end of body, outside or surrounding intestinal caeca. Excretory opening slightly dorsal at posterior end; excretory bladder with a very small median stem bifurcating into two long narrow ducts, one on each side of body outside caeca. Glandular vesicle in the form of a tubular mass usually present near hinder end behind ovary and genital pore.

Habitat—Ventricle of heart and main arteries.

Host—Water tortoises, *Kachuga dhongoka* and *Hardella thurgi*.

Locality—Allahabad, India.

*Type species*—*Plasmiorchis orientalis* sp. n.

#### Remarks on the Systematic Position of the Genus *Plasmiorchis* N. G., the Synonymy of Certain Genera and Classification of the Family Spirorchidae.

The genus *Plasmiorchis* belongs to the family Spirorchidae and the subfamily Spirorchinae, bearing a close relationship to the genus *Spirorchis* MacCallum 1918. It resembles the latter genus in the position and large number of testes, which lie in a linear series in the median plane, in the intracaecal area in front of the ovary and the genital pore. The vas deferens is absent, vesicula seminalis well developed and the cirrus sac small with poorly developed musculature as in *Spirorchis*. In *P. hardellii*, however, the vesicula seminalis is small and the cirrus sac relatively larger with strongly developed musculature. The ovary is always lobed and occupies the same position in the two genera, *i.e.*, median or slightly to the right, a little behind the hindmost testis. The size and position of the receptaculum seminis, uterus, vitelline ducts and vitelline reservoir are similar. The ovum is discharged singly and is without filaments. The vitellaria are voluminous and lateral; there is a close resemblance in the excretory system of the two genera. The glandular vesicle is also present near the hinder end of the body in both. The important features in which *Plasmiorchis* differs from *Spirorchis* are the presence of a ventral sucker (ventral sucker though absent in adult *P. pellucidus* is represented by a definite zone, the ventral sucker area) and forwardly directed loops at the origin of the intestinal caeca, one on each side of the oesophagus. It also differs in certain minor characters such as the

more forward extension of the vitellaria, which commence from about the middle of the oesophagus and the position of the genital opening, which lies a little behind the ovary and not in level with its caudal margin as in *Spirorchis*. The left intestinal caecum in *Plasmiorchis* gives off a loop, the genital loop in the region of the genital opening enclosing a space in which the cirrus sac and metraterm lie.

The genera *Henotosoma* Stunkard, 1922 and *Haematotrema* Stunkard, 1923 should be considered synonymous with the genus *Spirorchis*, which they resemble in the entire anatomy and topography of organs. The testes in these genera are large in number, and arranged in a linear series as in *Spirorchis*. The position of the ovary and the genital opening is also similar. The intestinal caeca arise just before the posterior end of the oesophagus and pass lateral about one half of the distance to the body wall, before they turn backwards, extending almost to the posterior end as in *Spirorchis*. They are also monostomes, lacking the ventral sucker. The only character in which they differ from the latter genus is the position of the testes in the posterior half of the worm. There are some minor points of difference between *Henotosoma* and *Haematotrema* in the number of testes and position of the genital pore; in the former the testes are ten in number, and in the latter four, situated in the anterior part of the posterior half of the body. The genital pore in *Haematotrema* lies a little in front of that in *Henotosoma*. In our opinion these differences are not important enough so as to be considered of generic rank, the genera *Henotosoma* and *Haematotrema*, therefore, are merged in the genus *Spirorchis*, the former reduced to *Spirorchis haematobium* (Stunkard, 1922) and the latter to *Spirorchis parvum* (Stunkard, 1923).

*Tremarhynchus indicus* Thapar, the account of which is published in June 1933 number of Helminthology, agrees closely in many features with the species of *Coeuritrema* described by me in May 1933. Both these genera possess two testes with the ovary between them, a large cirrus sac with the vesicula seminalis outside it, in front of the anterior testis and an eversible cirrus. The position of the genital pore in both the genera is sinistral and dorsal, close behind the ventral sucker near the middle of the body length. The important points of difference are the absence of receptaculum seminis, presence of shell glands and larger size of vitellaria in *Tremarhynchus*. Thapar makes no mention of the presence of salivary gland cells and metraterm in his species. The salivary gland cells are present without exception in all the families of blood flukes and it is impossible to believe that they are absent in *Tremarhynchus*. It appears likely that the vitellaria in the latter do not extend in front of the intestinal bifurcation and the salivary gland cells surrounding the oesophagus have been taken for the vitelline gland cells in that region. *Tremarhynchus indicus* resembles *Coeuritrema lyssimus* and *Coeuritrema odhnerensis* so closely that it is unlikely that the receptaculum

seminis, which is a characteristic feature of the latter two species, is absent in it. We also feel doubtful for the same reason about the existence of the shell gland cells and the absence of the metraterm in this species. Thapar's observation that in his species the two caeca run backwards to the posterior end as slender straight tubes except a little distance in front of the hinder end needs confirmation, because obviously the dorsal shifting of the genital pore in *Hapalorhynchus* and *Coeuritrema* has resulted in the formation of the characteristic loop of the left caecum. towards the median line in the region of the pore. Apart from these differences *Tremarhynchus indicus* differs from the species of *Coeuritrema* in minor features of a specific nature such as the size and shape of the body, size of the suckers, size and shape of the testes, ovary, vesicula seminalis and cirrus sac. In view of the foregoing, *Tremarhynchus indicus* must be considered synonymous with *Coeuritrema* and included in the latter genus on the basis of priority under the name of *Coeuritrema indicus* (Thapar). The latter species resembles *C. odhnerensis* in the shape of its body, but it is about twice in length of the latter species, and also differs in the oral sucker being larger than the ventral sucker, a feature in which it resembles *C. lyssimus*. The testes in *C. odhnerensis* are not so deeply lobed as in *C. indicus* in which they are described as divided into follicles, though Thapar's figure shows them to be deeply lobed and not separated into pieces so as to deserve the name of follicular testes, as the term is ordinarily used. The vitellaria in both these species extend a little more forwards than in *C. lyssimus*, i.e., in front of the ventral sucker as far as the intestinal bifurcation. *C. indicus* differs remarkably from the other species not only in the shape of its testes, but it also differs in the large size and position of its vesicula seminalis. The receptaculum seminis as mentioned above is presumed to be present in this species.

#### Family Spirorchidae Stunkard, 1921.

Stunkard gave a classification of this family in 1921, creating the subfamilies Spirorchinae and Hapalotreminae. In 1923 he gave a fuller account of the family assigning *Hapalotrema* Looss, 1899 and *Hapalorhynchus* Stunkard, 1922 to the subfamily Hapalotreminae and *Spirorchis* MacCallum, 1918, *Henotosoma* Stunkard, 1922 and *Haematotrema* Stunkard, 1923 to the subfamily Spirorchinae. The genera *Henotosoma* and *Haematotrema* are now held, as mentioned above, to be synonymous with the genus *Spirorchis*. Since 1923 many genera have been created and added to this family; it, therefore, appears necessary to revise the family and subfamily diagnosis, and give keys for the identification of the genera and species.

*Family diagnosis.*—Small delicate hermaphrodite blood flukes with poorly developed musculature; monostomes or distomes. Pharynx absent;

oesophagus long, surrounded by salivary gland cells, which are numerous near its posterior extremity; intestinal caeca ending blindly near posterior end, with or without forwardly directed loops at their origin; only one intestinal caecum present in *Unicaecum*. Genital opening sinistral, dorsal or ventral, about middle of body length or near hinder end. Testes two with ovary between them (*Hapalorhynchus*, *Coeuritrema*), divided into a large number of follicles forming two masses, one in front of and other behind ovary (*Hapalotrema*), one large undivided testis behind ovary (*Vasotrema*) in front of ovary (*Unicaecum*), divided into follicles all arranged in a linear series anterior to ovary (*Spirorchis*, *Plasmiorchis*) or last one or two follicles behind ovary (*Diarmostorchis*, *Spirhupalum*). Ovary usually lobed, median, to right or left side, a little behind middle, or near hinder end of body, or long and rolled in posterior part of body (*Unicaecum*); receptaculum seminis and Laurer's canal present or absent. Cirrus sac small, well developed or rarely absent (*Hapalorhynchus*); vesicula seminalis externa large; protrusible cirrus well developed in some genera. Uterus short; metraterm poorly or strongly developed; ovum large with or without polar filament or filaments, discharged singly. Vitellaria lateral and extensively developed. Excretory vesicle small, dividing almost immediately into lateral ducts. Parasites in blood of turtles.

**Type genus.**—*Spirorchis* MacCallum, 1918 (Syn. *Proparorchis* Ward, 1921).

#### Key to the subfamilies of Spirorchidae.

- |   |                       |
|---|-----------------------|
| 1. Genital pore and ovary near middle of body length.   | <i>Hapalotreminae</i> |
| Genital pore and ovary near hinder end . . . . .  | 2                     |
| 2. Testes arranged in a linear series all or except last one or two in front of ovary; two intestinal caeca present . . . . . | <i>Spirorchinae</i>   |
| Testis one continuous lobed structure and not divided into follicles; one intestinal caecum present . . . . .                 | <i>Unicaecumiinae</i> |

#### Subfamily Hapalotreminae Stunkard, 1921.

*Subfamily diagnosis.*—Spirorchidae: Genital pore sinistral, dorsal rarely ventral, near middle of body length. Testes two with ovary between them (*Hapalorhynchus*, *Coeuritrema*), divided into two masses of follicles one in front and other behind ovary (*Hapalotrema*) or only one large postovarial testis present (*Vasotrema*). Ovary lobed, faintly lobed, or entire, slightly to left side, near genital pore and middle of body length. Cirrus sac well developed, absent only in *Hapalorhynchus*, in front of ovary or anterior testis. Vesicula seminalis large, outside cirrus sac; protrusible cirrus present, absent only in *Hapalorhynchus*. Receptaculum seminis and Laurer's canal present. Vitellaria well



developed extending from intestinal bifurcation or behind ventral sucker to caudal end of caeca. Parasites of turtles

**Type genus.**—*Hapalotrema* looss, 1899.

**Key to the genera of the subfamily Hapalotreminae**

- |   |                       |
|---|-----------------------|
| Testes divided into two masses, one in front of and |                       |
| other behind ovary . . . . .                        | <i>Hapalotrema</i>    |
| Testes only two with ovary between them . . . . .   | 1                     |
| 1. Cirrus sac and cirrus present . . . . .          | <i>Cheuritrema</i>    |
| Cirrus sac and cirrus absent . . . . .              | <i>Hapalorhynchus</i> |
| Testis only one, behind ovary . . . . .             | <i>Vasotrema</i>      |

**Key to the species of the genus *Cheuritrema***

- |   |                      |
|---|----------------------|
| Ventral sucker smaller than oral sucker . . . . .   | <i>C. odnerensis</i> |
| Ventral sucker larger than oral sucker . . . . .    | 1                    |
| 1. Body narrow, pointed at hinder end; body length  |                      |
| 3'16—3'45; vesicula seminalis large, 0'2 in length  | <i>C. indicus</i>    |
| Body broad, somewhat rounded at hinder end; body    |                      |
| length 1'5—2; vesicula seminalis small, 0'048—0'057 |                      |
| in length . . . . .                                 | <i>C. lyssinus</i>   |

**Subfamily Spirorchinae Stunkard, 1921.**

*Subfamily diagnosis.*—Spirorchidae: Genital pore sinistral, ventral, near hinder end of body. Testes, large in number, arranged in a linear series in the intracaecal area all or except last one or two in front of ovary. Ovary lobed, dextral or median, near genital pore and hinder end of body. Cirrus sac small, with poorly developed musculature except in *Spirhapalum* and *Plasmiorchis hardellii*. Vesicula seminalis large, outside cirrus sac, with anterior end broad and in contact with hindmost testis. Receptaculum seminis and Laurer's canal present. Vitellaria both extra and intracaecal, extending from intestinal bifurcation or about middle of oesophagus to caudal end of caeca. Parasites of turtles.

**Type genus.**—*Spirorchis* Mac Callum, 1918 syn *Proparorchis* ward, 1921.)

**Key to the genera of the subfamily Spirorchinae.**

- |  |                          |
|--|--------------------------|
| Intestinal caeca with loops at their origin, one on      |                          |
| each side of oesophagus . . . . .                        | <i>Plasmiorchis</i> N.G. |
| Intestinal caeca without loops at their origin . . . . . | 1                        |
| 1. Testes all in front of ovary . . . . .                | <i>Spirorchis</i>        |
| One or two testes behind ovary . . . . .                 | 2                        |
| 2. Ventral sucker present; cirrus sac spacious . . . . . | <i>Spirhapalum</i>       |
| Ventral sucker absent; cirrus sac poorly developed       | <i>Diarmostorchis</i>    |

**Key to the species of the genus *Spirorchis*.**

- |  |   |
|--|---|
| Testes commencing behind middle of body . . . . .      | 1 |
| Testes commencing in front of middle of body . . . . . | 2 |

1. Testes 4 or 5 in number ; genital pore at one fourth body length from posterior end . . . . . *S. parvum*  
(Stunkard, 1922)
- Testes 10 in number ; genital pore near posterior end . . . . . *S. haematobium*  
(Stunkard, 1923)
2. Genital pore one seventh of body length from posterior end . . . . . 3
- Genital pore one fourth of body length from posterior end . . . . . 4
3. Testes larger than ovary, not distinctly separated . . . *S. innominata*
- Testes smaller than ovary, distinctly separated . . . *S. artericola*
4. Testes large, commencing immediately behind intestinal bifurcation . . . . . *S. scripta*
- Testes large, commencing some distance behind intestinal bifurcation . . . . . *S. elegans*
- Testes small, not more than one half size of ovary . . . *S. picta*

#### Key to the species of the genus *Plasmiorchis*

- Ventral sucker absent in adult, but ventral sucker area present . . . . . *P. pellucidus*
- Ventral sucker present . . . . . 1
1. Cirrus sac larger, with stout musculature . . . . . *P. hardellii*
  - Cirrus sac very small, with weak musculature as in *Spirorchis* . . . . . 2
  2. Testes 5—7 in number ; caeca straight without outgrowths . . . . . *P. orientalis*
  - Testes much larger in number ; caeca undulating near posterior end and with small outgrowths . . . . . *P. obscurum*

#### Subfamily Unicaecuminae, new subfamily

*Subfamily diagnosis.*—Spirorchida :—Only one intestinal caecum present, the other atrophied and absent. Testis one continuous lobed structure, and not divided into separate follicles; vas deferens arises from anterior end of testis and not from posterior extremity as in Spirorchinae; vesicula seminalis very long, spirally coiled and parallel to testis throughout length of body. Ovary long and coiled in posterior part of body. Parasitism of turtles.

**Type genus.**—*Unicaecum* Stunkard, 1927.

#### Discussion on the Relationships of the Families of Blood Flukes.

The relationships of the Spirorchidae with the Schistosomatidae have been discussed already (1933). The subfamily Hapalotreminae represents the ancestral blood flukes, from which have been evolved along one line the Schistosomatidae, and along another the degenerate suckerless blood flukes of the families Aporocotylidae and Sanguinicolidae. It is this latter part of the theme which we discuss further in this paper.

The highly interesting genera of blood flukes *Aporocotyle*, *Psettarium*, *Deontacylix* and *Sanguinicola* are unique among the Digenetic trematodes in having lost both the suckers. *Aporocotyle* was discovered by Odhner in 1900 as an ectoparasite from the gills of flounder. It was later announced by him in 1911 as a blood fluke. *Sanguinicola* was discovered by Plehn in 1905 as an endoparasitic Turbellarian, and included by her in a new family Rhynchostomida. Later in 1908 she considered it as a monozoic Cestode. Lühe in 1910 created for it a separate order of Cestoda, Rhynchostomida M. Plehn. Odhner in 1911 after comparing it fully with *Aporocotyle* and *Haplotrema* recognised its true nature as a Digenetic trematode. He also reported in this paper that the suckerless *Deontacylix ovalis* Linton from the intestine of a West Indies fish may be a blood fluke, which in reality is a relation of *Sanguinicola*, and in regard to the structure of the gut occupies an intermediate position between it and *Aporocotyle*. In 1912 Odhner created for the blood parasites of *Aporocotyle*-*Sanguinicola* series a new family Aporocotylidae, which was accepted and defined by Stunkard in 1923. Woodland in 1923, on the basis of his erroneous description of the genital organs of *Sanguinicola* in the blood of Siluroids from Sudan, agreed with Plehn's first idea of regarding that genus as an aberrant and much modified Turbellarian, and denied entirely its Malacocotylean affinities. Odhner in 1924 pertinently corrected this idea after giving a correct description of the African species, which he named *S. chalmersi*. The morphology and development of the European species of *Sanguinicola* have been recently investigated by Ejsmont in 1926. Poche in 1925 and Fuhrmann in 1930 accepted the family Aporocotylidae for *Aporocotyle* and *Deontacylix* and the family Sanguinicolidae for *Sanguinicola* in the Digenea. The latter family was created by Graff in 1907, when *Sanguinicola* was included in the Turbellaria. \*Rasin in 1929 assigned a new genus *Janickia* to the Sanguinicolidae, to which has also been added by Van Cleave and Mueller in 1932 a new species *Sanguinicola occidentalis* obtained from the heart of *Stixostedion vitreum* from Oneida Lake. Layman in 1930 described a new species *Aporocotyle odhneri* in blood of *Spheroides borealis* from the Sea of Japan. In 1929 Goto and Ozaki discovered in the intestine of a puffer, *Plehnia japonica* which they called *Psettarium japonica* in 1930. The latter species, which is considered to be closely related to *Deontacylix* appears to be a blood fluke. As its discoverers had apparently recourse to only one specimen for description, its true habitat in mesenteric blood vessels might have escaped their notice. Their statement that no blood corpuscles were found in the intestinal contents of the parasite does not seem to be a valid argument for denying its haematic abode, as Plehn in her first description had also stated that blood corpuscles were never found in the gut of *Sanguinicola*.

\* Biol. Spisy. Brno. 8, XVI, 1929.

From the account of morphology it is clear that we have a descending series in the evolution of the alimentary system of the four suckerless trematodes hitherto known, *Aporocotyle*—*Psettarium*—*Deontacylix*—*Sanguinicola*. In all these genera the gut is of the basic H-shaped type. In *Aporocotyle* the oesophagus is of about the same length as in the Spirorchidae and Schistosomatidae; it is also surrounded as in these families by the salivary gland cells. The intestinal caeca are also of the same length and reach near the hind end. The only point of difference, however, is the presence of anterior blind sacs in *Aporocotyle*. The intestinal caeca of *Plasmiorchis* have got forwardly directed loops at their origin exactly in a similar position to that of the anterior blind sacs of the gut of the latter genus. There is a peculiar similarity of the gut in this feature in the two genera. We may say that the formation of loops by the caeca at their origin as in *Plasmiorchis* provides an appropriate condition for the origin of the blind sacs at the anterior ends of the caeca of *Aporocotyle*. From this it follows that *Plasmiorchis* is related to the ancestral blood fluke from which *Aporocotyle* is evolved. It appears that the evolution in the blood flukes, as in the rest of the Digenea, has taken place by mutations as displayed by certain basic tendencies. Just as an ancestral form like *Plasmiorchis* came into existence with loops at the origin of the caeca to provide for an increased absorptive surface for food, another form closely related to it arose in the Hapalotreminae having two limbs of the loop fused, as it were, to form the anterior blind sac of one side of the gut. From the gut of *Aporocotyle* can be derived that of *Psettarium* in which the oesophagus is long and opens in the centre of the H-shaped intestine. The anterior caeca are small, i.e., about one third as long as the oesophagus, but the posterior caeca are much smaller than those of *Aporocotyle*, terminating much in front of the hinder end. From *Aporocotyle* onwards there is a great tendency in this series of genera towards a reduction in the length of the intestinal caeca and from *Psettarium* onwards there is also a tendency towards a greater development of the anterior horns. This has culminated in the extremely small H-shaped gut of *Sanguinicola*, in which both the anterior and posterior horns are of nearly equal size. *Deontacylix*, in this respect, occupies an intermediate position between *Psettarium* and *Sanguinicola*. It has posterior caeca much smaller and anterior caeca much larger than those of *Psettarium*, but they are both of nearly equal size as in *Sanguinicola*, though much larger than in the latter genus. Goto and Ozaki do not mention the presence of salivary gland cells in *Psettarium*, but we presume that they are present in this genus also. In *Sanguinicola chalmersi* the lobes of the H-shaped gut have lost their separate entity and the gut has consequently taken an irregular shape of the Rhabdocoele type. The mouth opening is extremely small in these blood-sucking genera.

There is a close similarity in the genital organs of these four genera. The testes in all of them are divided into a large number of follicles. It has been suggested in a previous paper that the ancestral blood fluke possessed two testes with the ovary between them like *Coeuritrema* and that the presence of a large number of testes in front of the ovary, which lies near the hind end as in the Spirorchinae and the Aporocotylidae, is a secondary condition evolved from the condition in the Hapalotreminae, in which the anterior testicular mass developed preponderantly, so that the ovary with its associated ducts and the genital pore came to lie near the hind end, whilst the posterior testis not possibly divided into follicles became entirely suppressed. In *Aporocotyle* the testes, large in number, occupy irregularly the entire intracaecal region between the intestinal bifurcation and the ovary; in the Spirorchinae they occupy the same position with this difference that their number is smaller and arrangement regular in a linear series. The former condition is primitive and the latter secondary. We are inclined to believe that *Aporocotyle* represents the origin of one-side branch and Spirorchinae as the other of the main stem represented by the common ancestor, in which the *Aporocotyle* arrangement of testes and ovary was present. In *Psettarium* and *Deontacylix* the testes lie both outside and inside the caeca, occupying nearly the entire space available, between the intestinal bifurcation and the ovary, on account of reduction in length of the caeca and their approachment towards each other near their origin. In *Sanguinicola*, however, their arrangement is somewhat regular in a double row, behind the gut and in front of the ovary, but this is obviously a departure from the irregular arrangement of *Aporocotyle* type along another direction from that, shown by the testes of *Psettarium* and *Deontacylix*. The shape of the ovary varies in different genera or even in different species of the same genus and should not be considered of much importance from the point of view of these relationships. It is spherical or ovoid in *Aporocotyle*, slightly lobed in *Deontacylix*, much lobed in several species of *Plasmiorchis* and *Spirorchis*, ramified or aciniform in *Psettarium* and H-shaped in *Sanguinicola*.

The genital pore or separate male and female openings in the suckerless genera lie dorsal to the left side (dorsal and nearly median in *Sanguinicola*) near posterior end. In the Hapalotreminae also the genital pore lies dorsal to the left side, though near the middle of the body length. This also supports the view mentioned above about the evolution of these forms from the Hapalotreminae. The Spirorchinae, in which the genital pore is ventral near the posterior end, represents obviously another line from the same common ancestor. The genus *Aporocotyle* among the genera of its own line stands closer to that ancestor, in that it has one opening for both male and female ducts, which lies in front of the ovary; whereas the other genera show a specialized condition in that the male and female openings are separate,

a condition which is not without its parallel in the Digenea. The cirrus sac in *Aporocotyle* is also fairly well developed, resembling that of the Haplotrematidae. Though it is smaller in *Psettarium* and *Deontacylix*, it is conical and bent as in the latter subfamily. A large vesicula seminalis outside the cirrus sac similar to that of the Spirorchidae is present in *Deontacylix*. The uterus in *Aporocotyle* and *Psettarium* is much larger than in the latter family consisting of a number of convolutions and containing a large number of ova. In *Deontacylix* it is much larger and filled with numerous ova. As in the Schistosomatidae the genus *Schistosoma* has secondarily developed a uterus containing a large number of ova, whereas its ancestors *Bilharziella*, *Ornithobilharzia*, *Austroilharzia* and *Heterobilharzia* have a very small uterus containing only one ovum, in the same way *Aporocotyle*, *Psettarium* and *Deontacylix* have secondarily acquired a large uterus. In this respect *Sanguinicola* with a very small uterus containing only one ovum shows the primitive condition. The metraterm is well developed in *Aporocotyle* as in many Haplotrematidae. The vitellaria are extensively developed in the latter genus as in the Spirorchidae. In *Psettarium* they are still more extensive, occupying the entire ventral surface of the body from the anterior end to the ovary. In *Sanguinicola* they are also extensive; in *S. chalmersi* they extend posteriorly even behind the ovary. There is only one vitelline duct present in all these genera except *Sanguinicola occidentalis* the left one having disappeared as pointed out by Odhner.

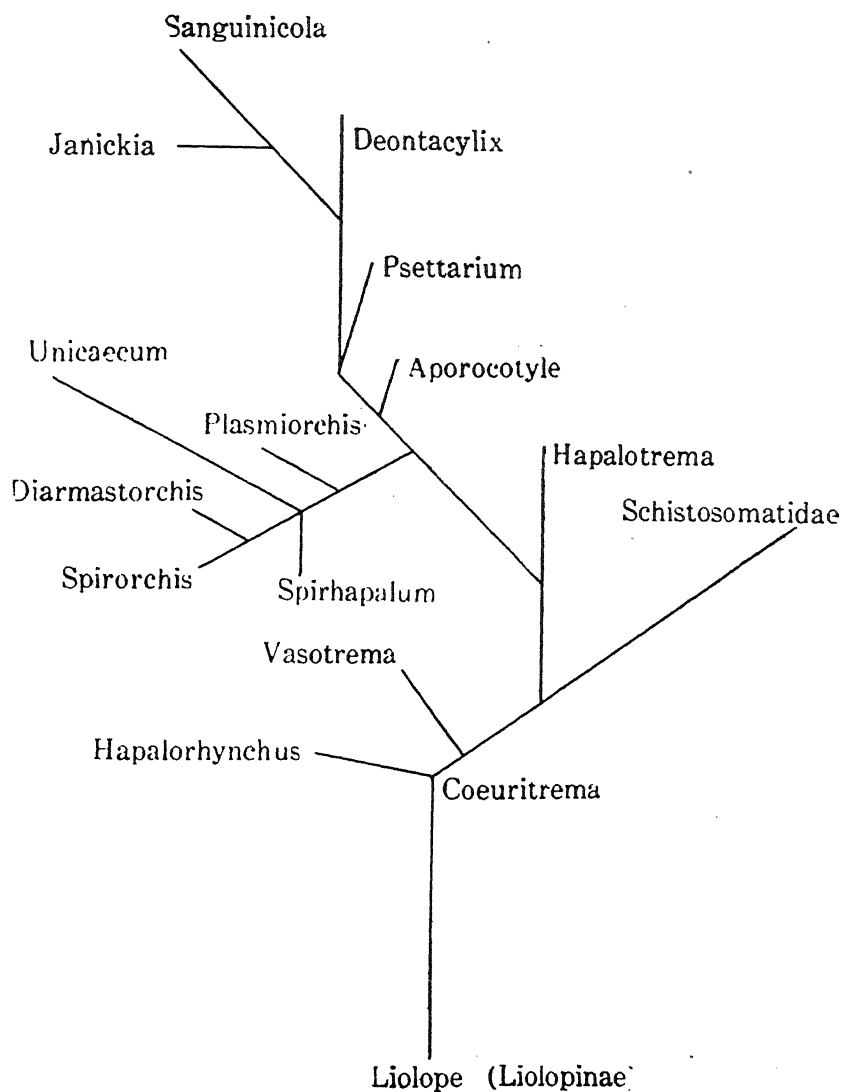
In the excretory, nervous and muscular systems also there is a substantial agreement not only between these four genera but also between them and the Spirorchidae. In the scheme of relationships of the blood-fluke families it appears certain, as shown above, that *Aporocotyle* stands near the Spirorchidae, and represents a close relation of the ancestor, from which are evolved along one line its closely related genera *Psettarium*, *Deontacylix* and *Sanguinicola* and along another the subfamily Spirorchinae. The genus *Unicaecum* has arisen as an aberrant branch from the latter subfamily, and we accordingly, include it in a new subfamily. *Aporocotyle*, *Psettarium* and *Deontacylix* are assigned to the family Aporocotylidae.

#### EXPLANATION OF THE PLATES.

- Fig. 1. Ventral view of *Plasmiorchis orientalis*.
- Fig. 2. Dorsal view of *P. pellucidus*.
- Fig. 3. Ventral view of *P. hardellii*.

#### Microphotographs

- Fig. 4. *P. orientalis*. Leitz Eyepiece X; Leitz Objective I.
- Fig. 5. *P. pellucidus*. Leitz Eyepiece X; Leitz Objective I.



Tree indicating the probable phylogeny of the blood flukes



- Fig. 6. *P. hardellii*. Zeiss Eyepiece X; Leitz Objective I.  
 Fig. 7. *P. obscurum*. Leitz Eyepiece X; Leitz Objective I.  
 Fig. 8. Immature specimen of *P. orientalis*. Leitz Eyepiece X; Leitz Objective I.  
 Fig. 9. T. s. of *P. orientalis* in the region of ventral sucker. Leitz Eyepiece 3; Leitz Objective 45 X.  
 Fig. 10. Hinder part of body of *P. pellucidus*. Zeiss Eyepiece X; Leitz Objective 3-10 X.  
 Fig. 11. Hinder part of body of *P. hardellii*. Leitz Eyepiece 3; Leitz Objective 3-10 X.  
 Fig. 12. Part of t. s. of *P. hardellii* in the region of cirrus sac. Leitz Eyepiece 3; Leitz Objective 3-10 X.  
 Fig. 13. T. s. of *P. hardellii* in the region of metraterm, cirrus sac and genital opening. Leitz Eyepiece 3; Leitz Objective I.

#### LETTERING.

a. l. i. c., anterior loop of intestinal caecum; c. s., cirrus sac; e. v., excretory vesicle; g. l. i. c., genital loop of intestinal caecum; g. o., genital opening; g. v., glandular vesicle; i. c., intestinal caecum; m., metraterm; o. s., oral sucker; oes., oesophagus; oes. v., oesophageal vesicle; ov., ovary; r. s., receptaculum seminis; t., testis; ut. uterus; v. s., ventral sucker; v. r., vitelline reservoir; v. s. a., ventral sucker area; v. sm., vesicula seminalis; vit., vitellaria.

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**Errata in Part I Published in *Bull Acad. Sc. U. P., Allahabad*. Vol 2,  
No. 4, May 1933:—**

Page 207, line 19 for "distel" read distal.

Page 209, line 2 from bottom add between "right intestinal caecum" and "by the intervening metraterm" the following, separated from the left caecum.

Page 213, line 3 after *Henotosoma* Stunkard for "1923" read 1922.

Page 213, line 9 for "*Haemato rema*" read Hapalotrema.

Page 213, line 22 for "Proparorchidae" read Proparorchis.

Page 221, line 12 for "Zoolopathologica" read Zoopathologica.

Page 221, line 17 for "Pein" read ein.

Page 221, line 19 for "(1921)" read (1912)

Plate I

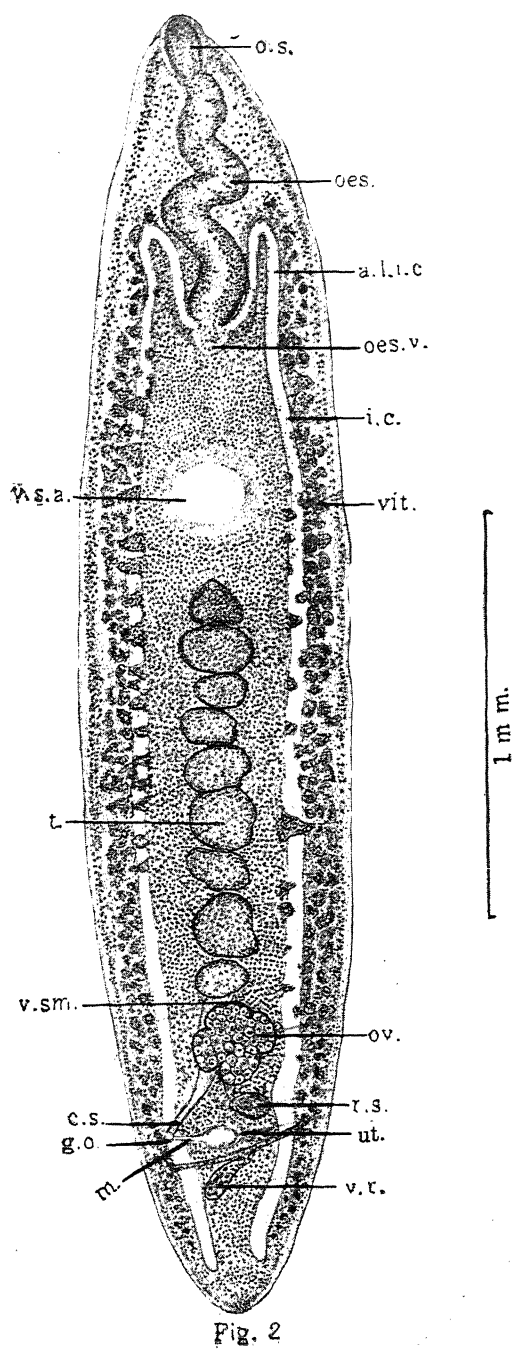
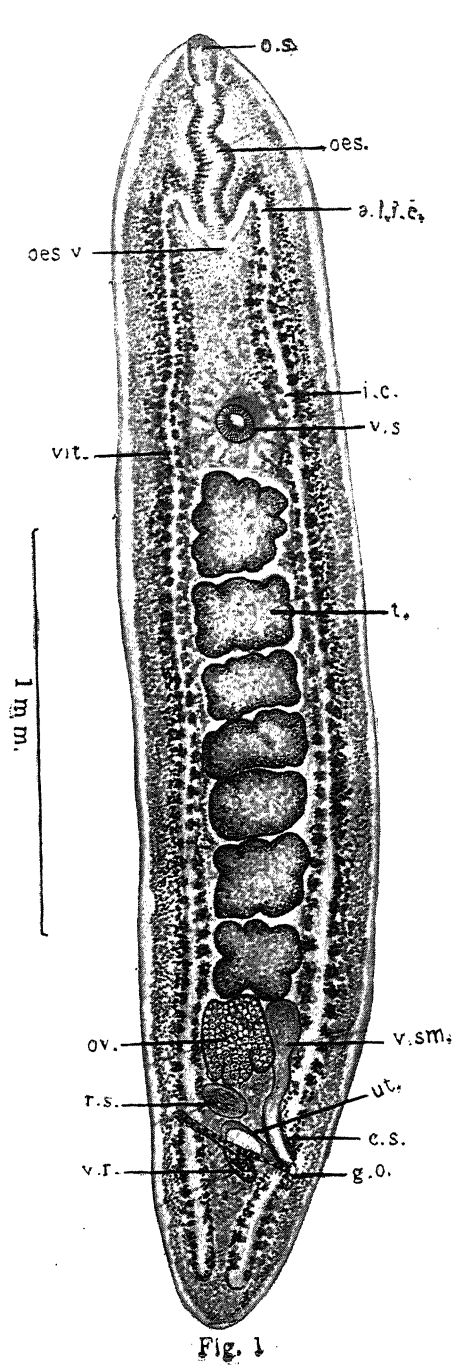




Plate II

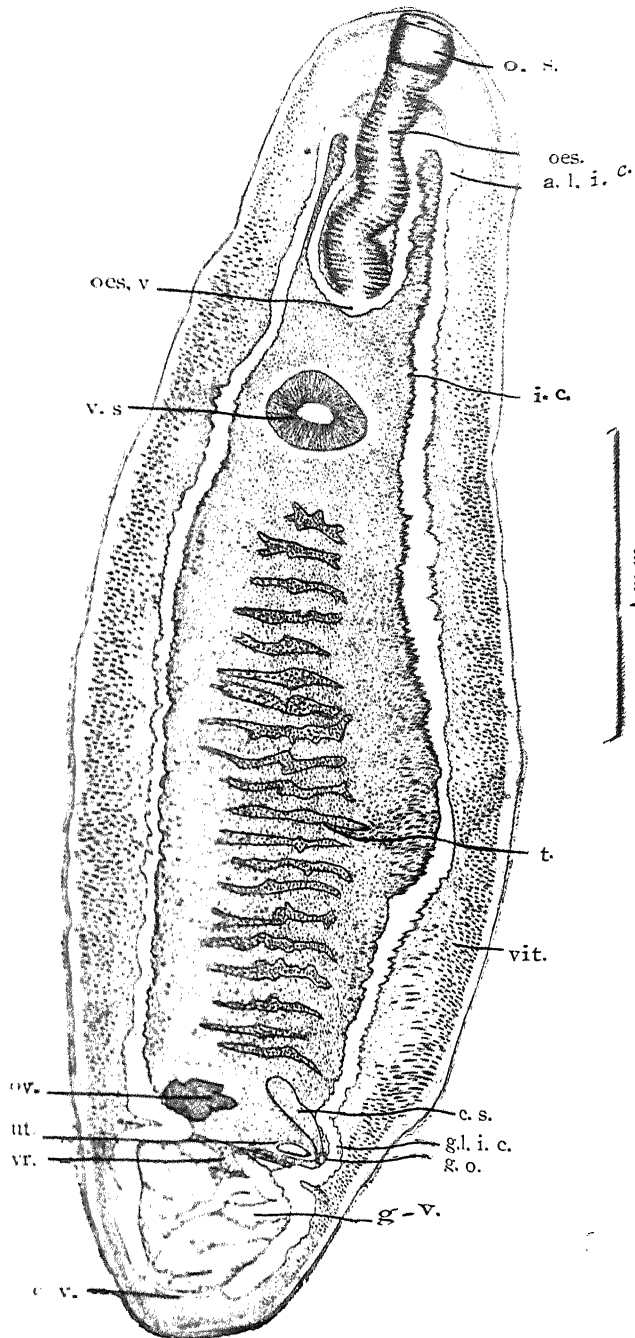


Fig. 3





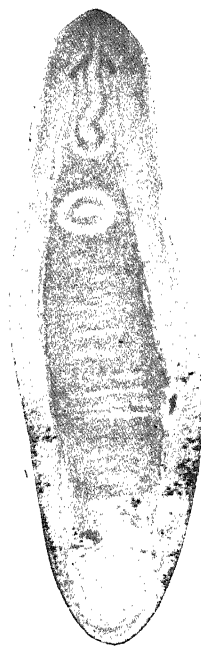
Plate III



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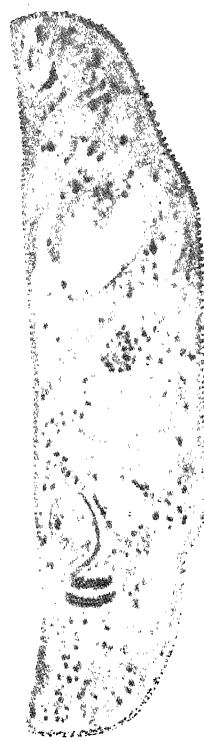
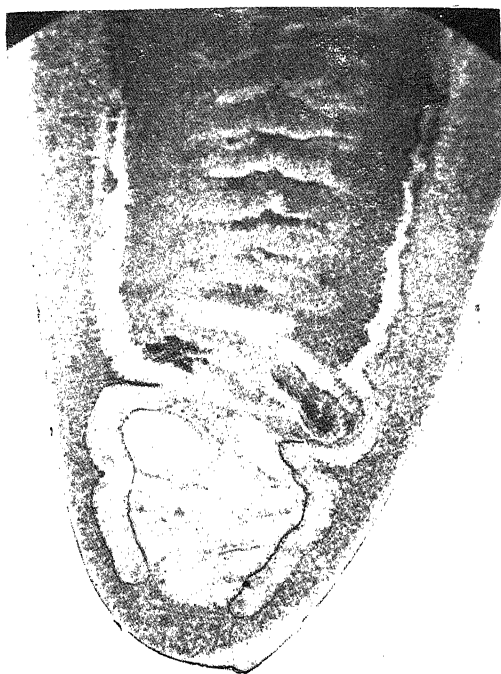
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Plate IV



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# ON THE ABSORPTION SPECTRUM OF NITROGEN MONOXIDE IN THE SCHUMANN REGION

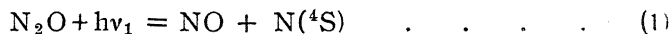
By P. K. SEN-GUPTA

DEPARTMENT OF PHYSICS, ALLAHABAD UNIVERSITY

Communicated by Prof. M. N. Saha

Received December 14, 1933

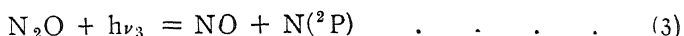
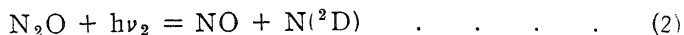
The absorption spectrum of Nitrogen Monoxide was studied by Leifson<sup>1</sup> in the Schumann region and by Datta<sup>2</sup> in the quartz region. Leifson used a vacuum grating spectrograph and absorption cells with fluorite windows and worked chiefly between  $\lambda 2400$  and  $\lambda 1240$ . The absorption column was 1.5 cms. in length with  $N_2O$  at atmospheric pressure. He found two continuous absorption bands, the first extending from  $\lambda 2000$  to  $\lambda 1680$ , and the second from  $\lambda 1550$  to the limit of fluorite. But no explanation of these cuts were offered. Six years later Wulf and Melvin<sup>3</sup> performed some irradiation experiments with  $N_2O$  and showed that it is photochemically decomposed by light of wavelength  $\lambda 2300$  into NO and N. In 1932 Datta again studied the absorption of  $N_2O$  in the quartz region, and tried to interpret his results from thermochemical data. He found  $N_2O$  to absorb light continuously from a long wavelength limit and traced it with the aid of some microphotograms to  $\lambda 2750$  which is much further on the long wavelength side than Wulf and Melvin's limit. Ascribing the beginning of absorption at  $\lambda 2750$  to the photodissociation of  $N_2O$  into NO and  $N(^4S)$ , as given in



Datta calculated the heat of dissociation of  $N_2$  with the aid of some thermochemical equations and obtained a value of 8.7 volts which agreed well with some other correct determinations of  $D_{N_2}$ . This proved the validity of assuming a process like (1).

At first sight this process seems to have no connection with Leifson's absorptions at  $\lambda 2000$  and  $\lambda 1550$ . In this connection attention may be drawn to the cases of  $SO_3^4$  and other higher oxides<sup>5</sup> and sulphides<sup>6</sup> and other

compounds in which a first absorption is generally followed by retransmitted patches of light with subsequent absorptions which correspond to liberation of atoms in metastable states. With  $\text{N}_2\text{O}$  a similar behaviour should be expected with a second and third setting of absorption according to the following equations,



where  $^2\text{D}$  and  $^2\text{P}$  are the metastable states of nitrogen. Taking Compton and Boyce's<sup>7</sup> value of  $^4\text{S}-^2\text{D}$  of nitrogen, Datta identified the second of Leifson's limits at  $\lambda$  1550 as indicating the decomposition of  $\text{N}_2\text{O}$  into NO and N in the  $^2\text{D}$ -state, on the grounds that with the aid of microphotograms  $\lambda$  1550 could be extended to  $\lambda$  1840 which is the expected place. The following work in the Schumann region was performed with a view to verify this point.

### EXPERIMENT

I have used a fluorite prism spectrograph designed according to our own directions. The spectrograph has strong light gathering powers and the region  $\lambda$  2200 to  $\lambda$  1300 comes on a plate 10 cms. long. It is specially designed for absorption work in the Schumann region. The absorption chamber which was of glass was separate and could be sealed to the end of the spectrograph carrying the fluorite window. The spectrograph and the absorption chamber were evacuated separately by common Holweck molecular pump and in the line a discharge tube was connected to test the vacuum. To the other end of the absorption chamber a hydrogen tube (run by a 2KW transformer) with a fluorite window was joined by means of a wide bore pressure tube. Thus the fluorite windows of the hydrogen tube and the spectrograph were common to the absorption chamber also. The spectrum of hydrogen served as a continuous source of light up to  $\lambda$  1600 after which the secondary emission spectrum of hydrogen appeared extending to the limit of fluorite. An idea of the vacuum in the chamber could be formed from the nature of the discharge in a vacuum tube attached to the spectrograph which was run by an induction coil.

It was found by trial that the length of the absorption chamber about 30 cms.) was too long for the present purpose, and therefore a separate absorption cell having a length of only 10 cms. had to be inserted into



Fig. 1

the main chamber. This cell carried as usual fluorite windows at the ends and

was also provided with a bent side tube so as to facilitate insertion in the main chamber. After the cell was filled with  $N_2O$  dried with  $P_2O_5$  at the requisite pressure the extreme end was sealed off. Fig. 2 shows the main absorption chamber and the subsidiary absorption cell in position. A

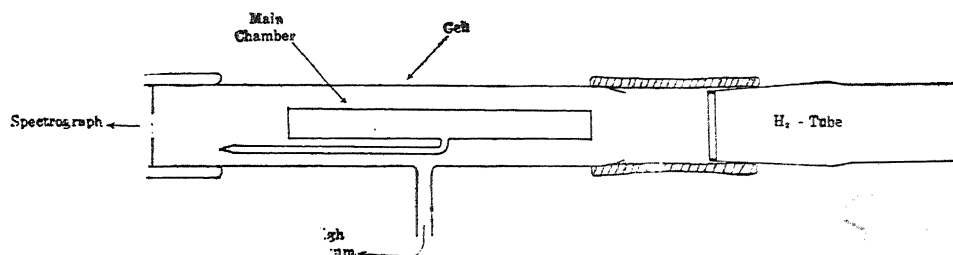


Fig. 2

series of different pressures were used ranging from 0.5 cm. to 20 cms. and photographs taken on Schumann plates.

The development of the absorption spectrum was as follows. At very low pressures light was cut off at about  $\lambda$  1850 and reappeared at about  $\lambda$  1700 and was again cut off at about  $\lambda$  1580. These absorptions were rather sharp in comparison to Datta's absorption in the quartz region. With increasing pressures the retransmitted patch of light from  $\lambda$  1700 to  $\lambda$  1580 vanished and only the absorption at  $\lambda$  1850 was visible. At still higher pressures the absorption at  $\lambda$  1850 began to extend towards longer wavelengths finally extending beyond  $\lambda$  2200. This was due to the fact that owing to heavy absorption, the retransmitted patches are wiped out.

#### DISCUSSION

The first beginning of absorption was found by Datta to correspond to  $\lambda$  2750. Now according to the second and third processes  $h\nu_1 - h\nu_2$  and  $h\nu_2 - h\nu_3$  should give approximate values of  $^4S - ^2D$  and  $^2D - ^2P$  of nitrogen, which, from the work of Compton and Boyce (loc. cit.) on the classification of arc spectrum of nitrogen are known to be 2.37 and 1.19 volts respectively. Since  $h\nu_1$  is equal to the energy corresponding to  $\lambda$  2750, that is, 4.53 volts,  $h\nu_2$  should be 6.9 volts corresponding to  $\lambda$  1800 and  $h\nu_3$  should be 8.1 volts corresponding to  $\lambda$  1540. In the present experiment the first beginning of absorption at  $\lambda$  1850 and the second at  $\lambda$  1580 no doubt correspond to  $h\nu_2$  and  $h\nu_3$  respectively. The experimental value, therefore, are—

$$h\nu_{2750} - h\nu_{1850} = ^4S - ^2D \text{ of N} = 2.17 \text{ volts,}$$

$$h\nu_{1850} - h\nu_{1580} = ^2D - ^2P \text{ of N} = 1.13 \text{ volts.}$$

In some of these cases it seems that molecular spectra yield low values of the difference of atomic terms—this phenomenon is yet not satisfactorily



explained. That the difference might not be the same as those obtained by the

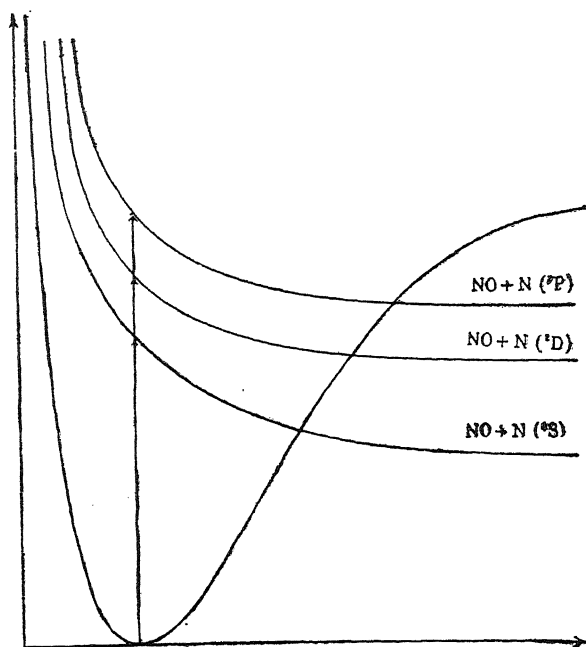


Fig. 3

arc spectrum could be understood from the adjoining potential energy diagram, since we do not know the nature of the upper curves. This point has been discussed in the paper on the sulphides of zinc, cadmium and mercury.<sup>8</sup>

Now it has already been mentioned that Datta identifies Leifson's absorption at  $\lambda$  1550 as due to the interaction of a nitrogen atom in the  $^2D$  state with NO. His argument is based on the grounds that since the first absorption at  $\lambda$  2750 is extended the second should also be extended; and as

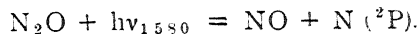
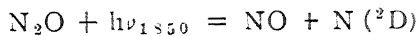
Leifson has no doubt given the values of complete extinction of light at  $\lambda$  1550, this could be traced to  $\lambda$  1840 nearly with the aid of microphotograms. It might be remarked here that from the appearance of an extended absorption as a first cut one cannot predict that the subsequent absorptions will be extended too. In the present experiment it has been found that the absorptions in the Schumann region are not at all extended and therefore it is highly improbable for a shift of about 290 Å to take place by microphotograms. Leifson's absorption at  $\lambda$  1550 obviously corresponds to an interaction of NO with a nitrogen atom in  $^2P$  state, and not  $^2D$  state as Datta holds. Leifson used a high pressure in his absorption cell, and this most probably accounts for the absence of the second absorption at  $\lambda$  1850. We generally come across such peculiar behaviour of gases at different pressures. It appears that the absorption at  $\lambda$  2000 obtained by Leifson is the same as  $\lambda$  1850 shifted towards longer wavelengths due to high pressure.

In conclusion the author gratefully acknowledges the valuable guidance rendered by Prof. M. N. Saha, D.Sc., F.R.S.

#### SUMMARY

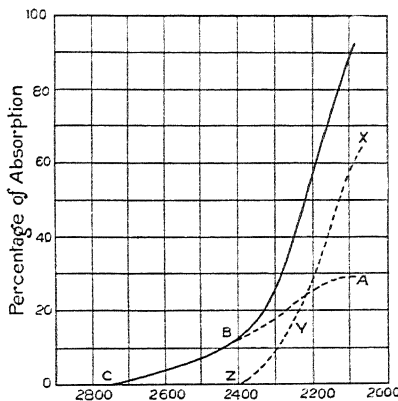
Absorption experiments with  $N_2O$  has been performed in the Schumann region at different pressures. It has been found that light is continuously cut off at  $\lambda$  1850, reappears at about  $\lambda$  1700 and is again cut off at  $\lambda$  1580. It is

suggested that the foregoing absorptions are due to photochemical dissociation of  $N_2O$  into NO and nitrogen in the metastable states according to the following—



#### SUPPLEMENT ADDED IN PROOF

On. of Datta's<sup>2</sup> curves for  $N_2O$  in the quartz region has been reproduced here. The point C corresponds to the long wave beginning of absorption which marks the photochemical dissociation of  $N_2O$  into NO and  $N(^4S)$ . But the curve has rather a peculiar shape. It is not smooth, but has a discontinuity at the point B. The author<sup>9</sup> has shown elsewhere that a discontinuity or Kink at any point of the absorption curve has a special significance. It has been shown that generally the main curve is not a single one but composed of two curves which have been drawn for  $N_2O$  here by dotted lines. In the figure these are ABC and XYZ which are quite distinct from each other.



What is the process of dissociation of  $N_2O$  when we get two beginnings of absorption corresponding to two curves? This means that there are two processes.

Light is thrown on this problem from a scrutiny of the structure of  $N_2O$ . Various experiments on Dielectric constant have shown that while some of the investigators have found the electric moment as zero, others find a small positive value for it. The following models of  $N_2O$  are possible—

- (1)  $N-O-N$  :—The bonds may be single or multiple. No electric moment is expected for such a model on account of symmetry.
- (2)  $N \equiv N=O$  :—This model will show electric moment.
- (3)  $\begin{array}{c} O \\ \diagup \quad \diagdown \\ N \quad N \end{array}$  :—The O—N bonds are similar, but electric moment is possible.

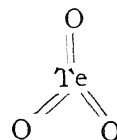
The study of absorption spectrum of  $N_2O$  definitely rules out the symmetrical rod model for it, as in this case there is only one type of bond to be severed. Corresponding to this splitting there will be only one absorption curve. The figure shows that there are two beginnings of absorption which

can only be explained if we suppose that there are two types of bonds present in  $N_2O$  having different energies, as in model 2 or 3.

The curve XYZ may be attributed to a splitting of the N—O bond, the energy of which is given by Z, that is,  $\lambda 2400 \equiv 5.2$  volts nearly. And ABC will correspond to the breaking of the N—N bond with energy given by C, that is,  $\lambda 2750 \equiv 4.5$  volts.

In  $CH_2Cl_2$ , by splitting the absorption curves N. K. Saha<sup>10</sup> first obtained evidence of the rupture of two types of bonds C—H and C—Cl, the difference in energy of which agreed remarkably well with that obtained thermochemically.

In  $TeO_3$  the structure is of the type shown from which we can expect only one smooth curve and no sign of kinks, as there is only one type of bond to be ruptured. This is borne out by experiment.<sup>5</sup>



In a recent paper,<sup>11</sup> Prof. Watson and his co-workers have given an account of the determination of the electric moment of  $N_2O$  and some other compounds. They have found a definite value for its electric moment which could not be decided by earlier measurements. The conclusion, they have drawn on the strength of their measurements regarding the structure of  $N_2O$  agrees with that arrived at in the present paper independently.

### References

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# ON THE ABSORPTION SPECTRA OF THE MONOXIDES OF THE ALKALINE EARTH METALS

By P. K. SEN-GUPTA.

PHYSICS DEPARTMENT, UNIVERSITY OF ALLAHABAD

Communicated by Prof. M. N. Saha.

Received March 5, 1934

It has already been shown by the author in several papers that the action of light on diatomic compounds of the oxide group is similar to that of the Alkali Halides, first investigated by Franck<sup>1</sup> and his co-workers. If we represent any of these compounds by MX where M is a divalent metal and X an element of the oxygen group, then on illumination of the vapour of the oxide by light of suitable frequency, the molecule is dissociated into its constituent atoms. The energy involved may be calculated from the beginning of the continuous absorption. There may be further cuts corresponding to the dissociation of the molecule into its excited atoms.<sup>2</sup>

The emission band spectra of the Monoxides of the Alkaline Earth metals were studied by Mecke,<sup>3</sup> Mahanti<sup>4</sup> and others, who have shown that the bands are due to a  $^1\Sigma \rightarrow ^1\Sigma$  transition. The present work deals with the absorption spectra of these oxides.

In a previous paper it has been shown that the oxides in the vapour state are polar compounds being formed of the ions  $M^{++}$  and  $X^{--}$  and it appeared from the interpretation of the absorption spectrum that light pulse as soon as it begins to be absorbed drives out both the electrons in  $X^{--}$  to  $M^{++}$  thus causing the oxide to be split up into M and X. In the present investigation I have tried to find whether the absorption by CaO, SrO and BaO can be interpreted in the same way.

## EXPERIMENT

It is well known that all these oxides are highly refractory. Mellor in his Treatise of Inorganic Chemistry writes, "..... W. R. Mott estimated that the boiling points of CaO about 3400°C and those of SrO and BaO occur respectively at 3000°C and 2000°C ...". For absorption it is not necessary that we should have vapour at sufficiently high pressure almost equal to that of the atmosphere, below which absorption can be detected. In fact, Claassen and Veenemans<sup>5</sup> have been able to determine the vapour pressures of the compounds between 1600—1750°K for CaO, 1500—1650°K for SrO, and 1200—1500°K for BaO, the temperatures being taken above the absolute zero. It has

been pointed out by many workers in the field that with very high temperatures the beginning of absorption is shifted towards longer wavelengths. To avoid errors due to shift, therefore, it was thought advisable to stick to the ranges given by Claassen and Veenemans as nearly as possible

The substances were vaporised in the vacuum graphite furnace of our laboratory<sup>11</sup> in the presence of nitrogen at atmospheric pressure. The column of vapour was nearly 15 cms. long in each case. Silica tubes were used to hold the substances. The source of light was a Hydrogen discharge tube run by a 2 KW transformer, and the copper arc was used for comparison. Photographs were taken by means of an  $E_3$  quartz spectrograph, on Schumann plates. To test the visible part of the spectrum a constant deviation spectrograph was used with either Process plates or Panchromatic plates, where necessary. Exposures were of the order of 2 to 3 minutes.

### RESULTS

In every case the spectrum was found to be continuously cut off from a long wavelength limit, and there was no trace of bands. To locate the beginning of the continuous absorption microphotograms were taken by the microphotometer belonging to the Physics Department, Muslim University, Aligarh. Here I must mention that I am very thankful to Dr. R. K. Asundi Reader in Physics there, for taking the microphotograms for me. On the same plate two exposures were given, that is, once the spot of light was allowed to run along the continuous spectrum and then the absorption spectrum.

Taking the ordinates of the continuous spectrum as 100 the percentage

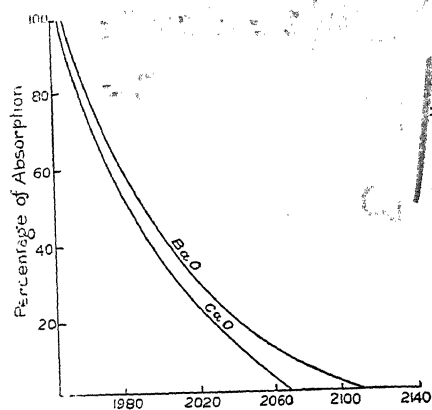


Fig. 1

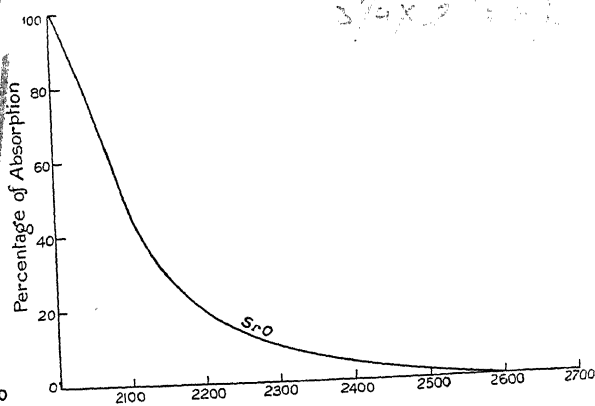


Fig. 2

of absorption was calculated from the absorption curves, and plotted with wavelength as abscissa. The point where such a curve meets the abscissa gives the beginning of absorption. From Figs. 1 and 2 we see that for CaO it is  $\lambda$  2070, for BaO  $\lambda$  2110, and for SrO  $\lambda$  2600 Å.

## CALCULATIONS

Taking  $R$  as the heat of dissociation of  $MX$  into  $M$  and  $X$  we get the following results from the Born Cycle, thermochemically.

Here  $L_{MX}$  is the Latent Heat of vaporisation of  $[MX]$ , and  $L_M$  that of  $[M]$   $D_{X_2}$  is the Heat of Dissociation of  $X_2$ .

Therefore,

$$R + L_{MX} = Q + \frac{1}{2}D_{X_2} + L_M$$

$$R = Q + \frac{1}{2}D_{X_2} + L_M - L_{MX}$$

The following table gives the values of the different quantities, the calculated values of  $R$  and the long wave limits of absorption from the graphs. The values of  $L_{MX}$  have been taken from Claassen and Veenemans' paper, and other data from Landolt and Börnstein and other tables.

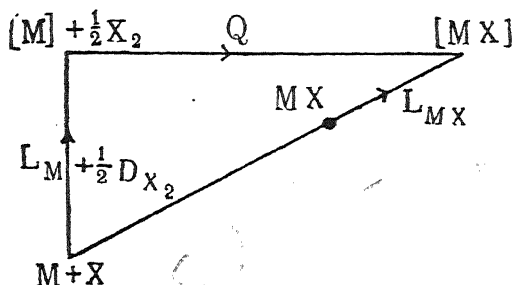


Table I

MX	$Q$ k. cal.	$\frac{1}{2}D_{X_2}$ k. cal.	$L_M$ k. cal.	$L_{MX}$ k. cal.	$R$ k. cal.	$h\nu$
CaO	145.0	64	44.2	120	133.0	$\lambda$ 2070
SrO	141.0	64	32.5	140	98.2	$\lambda$ 2600
BaO	125.9	64	32.4	90	132.3	$\lambda$ 2110

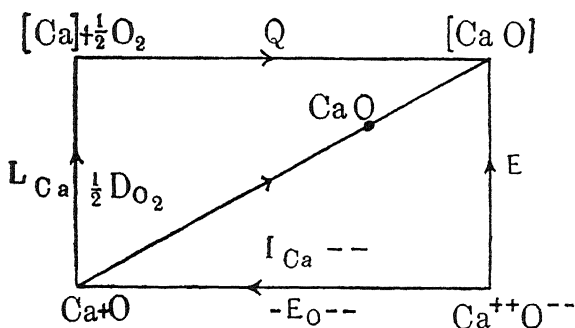
Now  $R = \frac{Nh\nu}{J}$ , and we see from Table I that there is fair agreement in the cases of CaO and BaO, but the wide divergence in the case of SrO leads one to doubt the correctness of the estimation of the latent heat of SrO. If we calculate indirectly from the long wave beginning of absorption, we find that  $L_{SrO} = 128$  k. cal.

## DISCUSSION

The appearance of the continuous absorption at the end of the spectrum in each case shows that the long wave limit of absorption is due to a transition from a firmly bound ground state to an unstable excited state. In such a case when no bands are present the binding in the ground state is supposed

to be of the ionic type. In the solid state CaO, SrO and BaO form ionic lattices of the type of NaCl. With this view in mind, therefore, we should assign such an electron structure to a compound like CaO which is also consistent with its diamagnetic behaviour. This is afforded with the structure  $\text{Ca}^{++}\text{O}^{--}$ . Here  $\text{Ca}^{++}$  and  $\text{O}^{--}$  both have the configuration  $[\text{Ar}]p^6$ , where  $[\text{Ar}]$  represents an inert gas core. The  $^1\text{S}_0$  state which results from this configuration contributes nothing to magnetism.

Taking  $\text{Ca}^{++}\text{O}^{--}$  as the normal structure of CaO in the ground state it is possible to calculate the energy required to dissociate CaO into Ca and O (normal, if the lattice energy is known. Born and Gerlach<sup>7</sup> calculated the value of the lattice energy in the following manner and applied it to the Born Cycle, to get the electron affinity of oxygen. Assuming the value of the electron affinity for oxygen we can utilise the Born Cycle to find the value of R.



Here  $I_{\text{Ca}^{++}}$  is the sum of the ionisation potentials of Ca and  $\text{Ca}^+$ ;  $E_{\text{O}^{--}}$  the heat of formation of an  $\text{O}^{--}$  ion out of O and 2 electrons.  $E$  the lattice energy. Hence,

$$R + L_{\text{CaO}} = E - I_{\text{Ca}^{++}} + E_{\text{O}^{--}}$$

The value of E has been calculated by Born and

Gerlach by means of the following formula—

$$E = k \cdot \frac{n-1}{n} \cdot \sqrt[3]{\frac{\rho}{M}}$$

$$= 2450 \cdot \frac{n-1}{n} \cdot \sqrt[3]{\frac{\rho}{M}}$$

where,

$k$  = a constant involving Madelung's coefficient,  
 $n$  = the repulsion exponent,  
 $\rho$  = the density,  
 $M$  = the molecular weight.

Here  $n$  is given by the formula,

$$n = 1 + C \cdot \frac{1}{\chi} \cdot \left(\frac{M}{\rho}\right)^{1/3}$$

where  $\chi$  is the compressibility.

For CaO, SrO and BaO,  $k=2450$ , but  $\chi$  is unknown for all these compounds. Born and Gerlach have taken a rough estimate of  $75 \times 10^{-12}$  which is a mean between  $77 \times 10^{-12}$  for ZnO and  $70 \times 10^{-12}$  for MgO.

Table II gives the values of the different quantities involved in calculating  $E$  and  $E'$  which is the value of the lattice energy calculated thermochemically from

$$E' = Q + L_{Ca} + \frac{1}{2}D_{O_2} + L_{Ca++} - E_O - -$$

Table II

Substance	I k. cal.	E k. cal.	M k. cal.	$\rho$	$n$	E k. cal.	E' k. cal.
CaO ...	411.7	49	56.1	3.25	5.93	800	714
SrO ...	383.0	49	103.6	4.34	9.12	758	670
BaO ...	348.2	49	153.3	5.30	11.40	728	620

It is evident that the values of lattice energy calculated from Born's equation (2) are higher than those calculated thermochemically from Born Cycle by 10 to 15 per cent. This leads to the conclusion that the assumption of approximate values of the compressibilities is wrong.

It is worth while mentioning at this place the extension made by Slater\* in the calculation of the lattice energies of alkali halides. Slater has determined the change of compressibility with temperature and extrapolated the values to the absolute zero. In some cases the values of the compressibility at absolute zero differ appreciably from those determined at room temperature, and, therefore, the energies of lattice will change a good deal. But according to Slater, the representation of the potential energy in Born's equation  $E = A + \frac{B}{r^n}$  by a single inverse term is erroneous. He, therefore, developed a series in terms of the change of compressibility with temperature and pressure, and recalculated the values of lattice energies which hardly differ from those obtained from Born's formula by 1 per cent. Since the dependence of compressibility on temperature has not been stated in a simple way, it is not possible to say whether Slater's values are better than those determined by Born's formula, as Hund has mentioned. Hence it is not possible to check the values of lattice energy unless an experimental determination is made of the compressibilities.

#### CONCLUSION

The calculations show that the behaviour of CaO, SrO and BaO in the presence of light is similar to those of the oxides and sulphides investigated



by the author. That is, the continuous absorption is due to the simultaneous transition of both the electrons in  $X^{--}$  to  $M^{++}$  so that two free and normal atoms are obtained.

*Possibility of a single electron transition.*—In the transition of a single electron from  $X^{--}$  to  $M^{++}$ , the resulting compound is  $M^+X^-$  in the process  $M^{++}X^{--} + h\nu = M^+X^-$ . The electrostatic attraction is still present. A transition  $M^{++}X^{--} \rightarrow M^+X^-$  should give bands but these were not obtained in the present work. It is quite possible that the short absorption column used in the present work was not effective in showing the bands in absorption. They might be present in the extreme infra-red or ultra-violet regions which will be investigated later on. The absence of the fundamental bands in emission of Mecke, Mahanti and others in the present absorptions shows that these bands do not correspond to transition to the fundamental level, but between two higher levels.

My sincerest thanks are due to Professor M. N. Saha, D.Sc., F.R.S., for valuable guidance and encouragement in connection with this work.

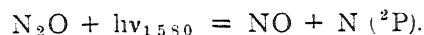
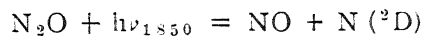
#### SUMMARY

1. The Monoxides of the Alkaline Earth Metals absorb light continuously from a long wavelength limit.
2. From thermochemical calculations according to Born Cycle it is postulated that the long wave limit of absorption corresponds to the energy required to dissociate the molecule into its constituent atoms in their normal states.

#### References

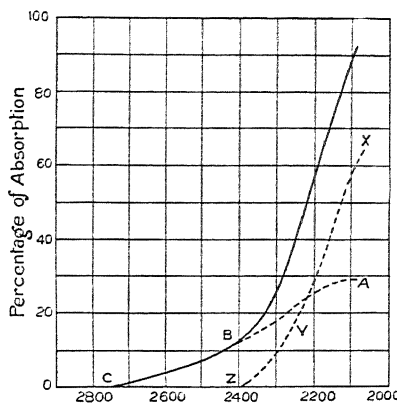
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8. Slater, *Phys. Rev.*, Vol. 23, p. 488, 1924.

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On. of Datta's<sup>2</sup> curves for  $\text{N}_2\text{O}$  in the quartz region has been reproduced here. The point C corresponds to the long wave beginning of absorption which marks the photochemical dissociation of  $\text{N}_2\text{O}$  into NO and  $\text{N}(^4\text{S})$ . But the curve has rather a peculiar shape. It is not smooth, but has a discontinuity at the point B. The author<sup>9</sup> has shown elsewhere that a discontinuity or Kink) at any point of the absorption curve has a special significance. It has been shown that generally the main curve is not a single one but composed of two curves which have been drawn for  $\text{N}_2\text{O}$  here by dotted lines. In the figure these are ABC and XYZ which are quite distinct from each other.



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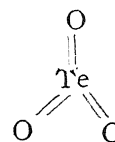
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The substances were vaporised in the vacuum graphite furnace of our laboratory in the presence of nitrogen at atmospheric pressure. The column of vapour was nearly 15 cms. long in each case. Silica tubes were used to hold the substances. The source of light was a Hydrogen discharge tube run by a 2 KW transformer, and the copper arc was used for comparison. Photographs were taken by means of an  $E_3$  quartz spectrograph, on Schumann plates. To test the visible part of the spectrum a constant deviation spectrograph was used with either Process plates or Panchromatic plates, where necessary. Exposures were of the order of 2 to 3 minutes.

### RESULTS

In every case the spectrum was found to be continuously cut off from a long wavelength limit, and there was no trace of bands. To locate the beginning of the continuous absorption microphotograms were taken by the microphotometer belonging to the Physics Department, Muslim University, Aligarh. Here I must mention that I am very thankful to Dr. R. K. Asundi Reader in Physics there, for taking the microphotograms for me. On the same plate two exposures were given, that is, once the spot of light was allowed to run along the continuous spectrum and then the absorption spectrum.

Taking the ordinates of the continuous spectrum as 100 the percentage

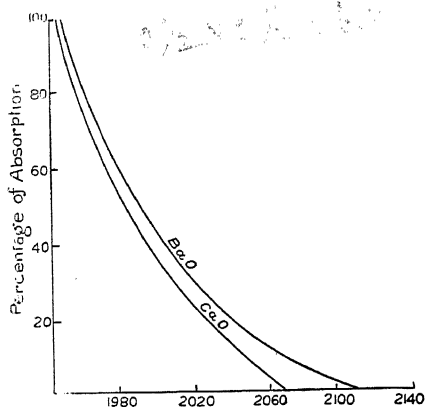


Fig. 1

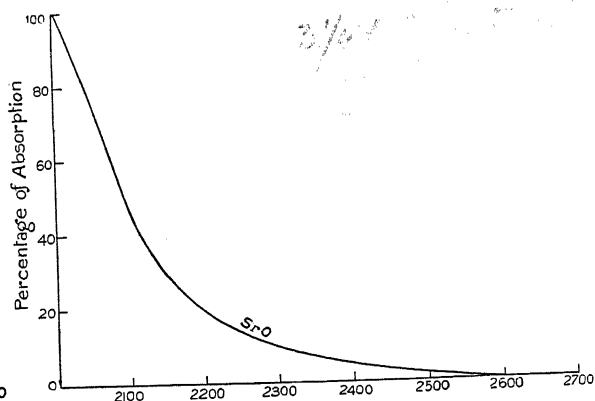


Fig. 2

of absorption was calculated from the absorption curves, and plotted with wavelength as abscissa. The point where such a curve meets the abscissa gives the beginning of absorption. From Figs. 1 and 2 we see that for CaO it is  $\lambda$  2070, for BaO  $\lambda$  2110, and for SrO  $\lambda$  2600 Å.

## CALCULATIONS

Taking  $R$  as the heat of dissociation of  $MX$  into  $M$  and  $X$  we get the following results from the Born Cycle, thermochemically.

Here  $L_{MX}$  is the Latent Heat of vaporisation of  $[MX]$ , and  $L_M$  that of  $[M]$   $D_{X_2}$  is the Heat of Dissociation of  $X_2$ .

Therefore,

$$R + L_{MX} = Q + \frac{1}{2}D_{X_2} + L_M$$

$$R = Q + \frac{1}{2}D_{X_2} + L_M - L_{MX}$$

The following table gives the values of the different quantities, the calculated values of  $R$  and the long wave limits of absorption from the graphs. The values of  $L_{MX}$  have been taken from Claassen and Veenemans' paper, and other data from Landolt and Börnstein and other tables.

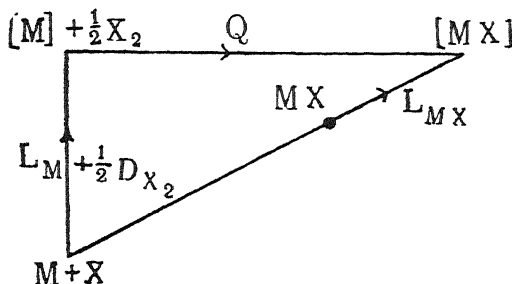


Table I

MX	$Q$ k. cal.	$\frac{1}{2}D_{X_2}$ k. cal.	$L_M$ k. cal.	$L_{MX}$ k. cal.	$R$ k. cal.	$h\nu$
CaO	145.0	64	44.2	120	133.0	$\lambda$ 2070
SrO	141.0	64	32.5	140	98.2	$\lambda$ 2600
BaO	125.9	64	32.4	90	132.3	$\lambda$ 2110

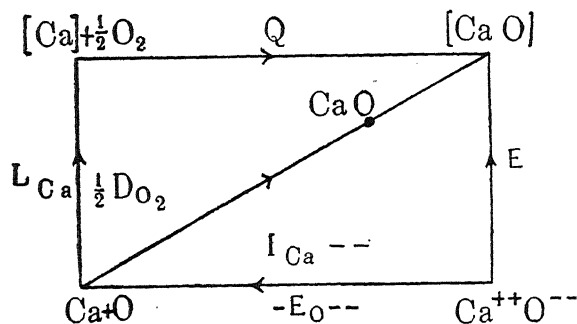
Now  $R = \frac{Nh\nu}{J}$ , and we see from Table I that there is fair agreement in the cases of CaO and BaO, but the wide divergence in the case of SrO leads one to doubt the correctness of the estimation of the latent heat of SrO. If we calculate indirectly from the long wave beginning of absorption, we find that  $L_{SrO} = 128$  k. cal.

## DISCUSSION

The appearance of the continuous absorption at the end of the spectrum in each case shows that the long wave limit of absorption is due to a transition from a firmly bound ground state to an unstable excited state. In such a case when no bands are present the binding in the ground state is supposed

to be of the ionic type. In the solid state CaO, SrO and BaO form ionic lattices of the type of NaCl. With this view in mind, therefore, we should assign such an electron structure to a compound like CaO which is also consistent with its diamagnetic behaviour. This is afforded with the structure  $\text{Ca}^{++}\text{O}^{--}$ . Here  $\text{Ca}^{++}$  and  $\text{O}^{--}$  both have the configuration  $[\ ]p^6$ , where  $[\ ]$  represents an inert gas core. The  $^1\text{S}_0$  state which results from this configuration contributes nothing to magnetism.

Taking  $\text{Ca}^{++}\text{O}^{--}$  as the normal structure of CaO in the ground state it is possible to calculate the energy required to dissociate CaO into Ca and O (normal, if the lattice energy is known. Born and Gerlach<sup>7</sup> calculated the value of the lattice energy in the following manner and applied it to the Born Cycle, to get the electron affinity of oxygen. Assuming the value of the electron affinity for oxygen we can utilise the Born Cycle to find the value of R.



Here  $I_{\text{Ca}^{++}}$  is the sum of the ionisation potentials of Ca and  $\text{Ca}^+$ ;  $E_{\text{O}^{--}}$  . . . the heat of formation of an  $\text{O}^{--}$  ion out of O and 2 electrons.  $E$  . . . the lattice energy. Hence,

$$R + L_{\text{CaO}} = E - I_{\text{Ca}^{++}} + E_{\text{O}^{--}}$$

The value of  $E$  has been calculated by Born and

Gerlach by means of the following formula—

$$E = k \cdot \frac{n-1}{n} \cdot \sqrt[3]{\frac{\rho}{M}}$$

$$= 2450 \cdot \frac{n-1}{n} \cdot \sqrt[3]{\frac{\rho}{M}}$$

where,  $k$  = a constant involving Madelung's coefficient,  
 $n$  = the repulsion exponent,  
 $\rho$  = the density,  
 $M$  = the molecular weight.

Here  $n$  is given by the formula,

$$n = 1 + C \cdot \frac{1}{\chi} \cdot \left(\frac{M}{\rho}\right)^{1/3}$$

where  $\chi$  is the compressibility.

For CaO, SrO and BaO,  $k=2450$ , but  $\chi$  is unknown for all these compounds. Born and Gerlach have taken a rough estimate of  $75 \times 10^{-12}$  which is a mean between  $77 \times 10^{-12}$  for ZnO and  $70 \times 10^{-12}$  for MgO.

Table II gives the values of the different quantities involved in calculating  $E$  and  $E'$  which is the value of the lattice energy calculated thermochemically from

$$E' = Q + L_{Ca} + \frac{1}{2}D_{O_2} + L_{Ca++} - E_O - -$$

Table II

Substance	I k. cal.	E k. cal.	M k. cal.	$\rho$	n	E k. cal.	E' k. cal.
CaO ...	411.7	49	56.1	3.25	5.93	800	714
SrO ...	383.0	49	103.6	4.34	9.12	758	670
BaO ...	348.2	49	153.3	5.30	11.40	728	620

It is evident that the values of lattice energy calculated from Born's equation (2) are higher than those calculated thermochemically from Born Cycle by 10 to 15 per cent. This leads to the conclusion that the assumption of approximate values of the compressibilities is wrong.

It is worth while mentioning at this place the extension made by Slater<sup>8</sup> in the calculation of the lattice energies of alkali halides. Slater has determined the change of compressibility with temperature and extrapolated the values to the absolute zero. In some cases the values of the compressibility at absolute zero differ appreciably from those determined at room temperature, and, therefore, the energies of lattice will change a good deal. But according to Slater, the representation of the potential energy in Born's equation  $E = A + \frac{B}{r^n}$  by a single inverse term is erroneous. He, therefore, developed a series in terms of the change of compressibility with temperature and pressure, and recalculated the values of lattice energies which hardly differ from those obtained from Born's formula by 1 per cent. Since the dependence of compressibility on temperature has not been stated in a simple way, it is not possible to say whether Slater's values are better than those determined by Born's formula, as Hund has mentioned. Hence it is not possible to check the values of lattice energy unless an experimental determination is made of the compressibilities.

#### CONCLUSION

The calculations show that the behaviour of CaO, SrO and BaO in the presence of light is similar to those of the oxides and sulphides investigated



by the author. That is, the continuous absorption is due to the simultaneous transition of both the electrons in  $X^{--}$  to  $M^{++}$  so that two free and normal atoms are obtained.

*Possibility of a single electron transition.*—In the transition of a single electron from  $X^{--}$  to  $M^{++}$ , the resulting compound is  $M^+X^-$  in the process  $M^{++}X^{--} + h\nu = M^+X^-$ . The electrostatic attraction is still present. A transition  $M^{++}X^{--} \rightarrow M^+X^-$  should give bands but these were not obtained in the present work. It is quite possible that the short absorption column used in the present work was not effective in showing the bands in absorption. They might be present in the extreme infra-red or ultra-violet regions which will be investigated later on. The absence of the fundamental bands in emission of Mecke, Mahanti and others in the present absorptions shows that these bands do not correspond to transition to the fundamental level, but between two higher levels.

My sincerest thanks are due to Professor M. N. Saha, D.Sc., F.R.S., for valuable guidance and encouragement in connection with this work.

#### SUMMARY

1. The Monoxides of the Alkaline Earth Metals absorb light continuously from a long wavelength limit.
2. From thermochemical calculations according to Born Cycle it is postulated that the long wave limit of absorption corresponds to the energy required to dissociate the molecule into its constituent atoms in their normal states.

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## CHEMICAL EXAMINATION OF THE BARK OF *NERIUM* *ODORUM*, SOLAND

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*Nerium Odorum*, Soland (N. O. Apocyanaceae) commonly known as Oleander in English, Kaner in Hindi and Karavi in Bengali and Sanskrit, is an ornamental garden plant cultivated throughout northern India. It is a well known poisonous plant of long use in Indian medicine. It has corked roots and thick and soft bark. The freshly cut bark exudes a pale yellow latex which on standing becomes resinous and sticky. It has long, slender, pointed leaves with red or white flowers. In the present investigation, the bark of the red variety was used.

As regards medicinal properties, both the root and the bark are highly useful. A decoction of the root is administered in a variety of fevers and the ground substance with water is applied in the form of a poultice on ulcers and boils. The bark is considered to be a powerful repellant and several preparations are made to be applied externally. It has been known for a long time that the active principle of *Nerium odorum* is a strong heart poison, producing powerful depression of the heart, and it is on this account that Prof. Pelikan suggested its use as a substitute for digitalis which is a well known heart depressant.

The bark of *Nerium odorum* was first examined by Greenish<sup>1</sup> and subsequently by Pieszezek<sup>2</sup> and Leulier.<sup>3</sup> They extracted from the bark a yellow aliphatic oil and a wax-like crystalline compound by petroleum ether, and two solid, bitter glucosides by alcohol. Greenish named the two glucosides as neriodorin and neriodorein. The authors did not go beyond studying the solubility of the compounds with different solvents and only the melting point of one of them is given as "above 56°". On the whole the statements of various authors regarding the active principle of the bark are very conflicting and no definite conclusion can be drawn from their work.

The present investigation was, therefore, undertaken with a view to subject the bark to a systematic chemical examination and determine its active constituents. As the result of that investigation it has now been

shown by actual isolation that the bark contains small quantities of a volatile essential oil, a yellow fixed oil, two amorphous glucosides, a solid crystalline wax, a phlobaphene, a tannin and a dark red colouring matter. The presence in the fresh bark of traces of peroxidase and a hydrolytic enzyme has also been shown.

#### EXPERIMENTAL

The dried and powdered bark was extracted with Prollius fluid and the extract tested with alkaloid reagents. A negative reaction indicated the absence of alkaloids. On ignition it left 13.5% of a white ash containing 78.0% of water soluble and 22.0% of water insoluble inorganic constituents. The soluble portion contained chlorides and sulphates of sodium and potassium together with traces of iron and the insoluble portion contained oxides of iron, aluminum and calcium together with comparatively large proportion of silica.

*Test for enzymes.*—The fresh bark on examination in the usual manner showed the presence of traces of a peroxidase (quinhydrone and purpurogallein reaction) and a hydrolytic enzyme (inversion of cane sugar). The absence of oxidase or reductase was also confirmed. In dried bark the enzymes become destroyed.

For complete analysis, 2 kilos of the dried and powdered bark were extracted with rectified spirit. The extract on cooling deposited a white flocculant precipitate which was filtered off (35 grams). The filtrate on concentration to a small volume under reduced pressure and allowing to stand deposited a sticky greenish yellow syrup from which the mother liquor was decanted off. The alcoholic mother liquor was then allowed to stand for a month pending the examination of other products.

*Examination of the white solid.*—The substance was thoroughly washed with chloroform and rectified spirit and then crystallised from boiling absolute alcohol in aggregates of colourless glistening prisms melting at 97°. The substance is sparingly soluble in cold alcohol, benzene, ether, chloroform, carbontetrachloride and petroleum ether, but dissolves to a moderate extent in hot alcohol, ethylacetate, acetone and pyridine. Concentrated sulphuric acid dissolves it with a yellow colour which slowly darkens and chars on warming. The substance was found to be a wax [Found: C=69.78, 69.84; H=11.40, 11.52%. M. W. (Cryoscopic in benzene)=461,473.] The wax had a sp. gr. of .9804, ( $d^{20}_4$ ) saponification value of 53.34, iodine value of 16.2, acid value of 16.8, and unsaponifiable matter of 62.4. The acid obtained from it by hydrolysis with alcoholic potash in the usual manner crystallised from alcohol in plates melting at 93-94°. This melting point agrees with that of cocceric acid, m. p. 92-93°. The alcohol obtained from the wax after hydrolysis by extraction with ether, was recrystallised from absolute alcohol in

flattened prisms melting at  $69^{\circ}$ . From the melting point as well as other properties the substance appears to be carnaubyl alcohol, m. p.  $68-70^{\circ}$ . Hence the wax obtained from the bark of the *Nerium odorum* appears to be the carnaubyl ester of cocceric acid.

*Examination of the greenish yellow syrup.*—This was submitted to steam distillation and the distillate extracted with ether. The extract on evaporation of the ether left a small quantity of an essential oil which had the characteristic odour of the drug. The quantity obtained, however, was too insufficient for any systematic examination.

The residue after steam distillation was extracted with petroleum ether and from the extract on complete evaporation of the solvent a yellow fixed oil was obtained which had properties similar to olive oil, but this was also too insufficient in quantity for systematic examination.

*Examination of the alcoholic mother liquor.*—The alcoholic mother liquor on being allowed to stand for about a month deposited a little more of the wax which was filtered. To the filtrate water was gradually added with stirring, when a brown resinous substance was precipitated. The precipitate was filtered off (20 gms.), washed thoroughly and dried.

The dark red filtrate was treated with aqueous lead acetate which caused the immediate precipitation of a yellowish grey substance. The lead compound was filtered off, washed with water and decomposed in aqueous suspension with hydrogen sulphide. After the removal of the lead sulphide the filtrate was evaporated to dryness. The substance thus obtained was an astringent, amorphous brown powder which answered most of the properties of tannins. Thus it dissolves in alkalies with a yellow colour and in concentrated sulphuric acid with a deep red colour. Ferric chloride gives a blue-black colour and precipitate. Lead acetate gives a light yellow, gelatine solution, a colourless and tartar-emetic, a light grey precipitate. The substance shrinks at  $210^{\circ}$  and melts with decomposition at  $240^{\circ}$ . It does not reduce Fehling's solution and is very soluble in water, alcohol, acetone and pyridine, sparingly soluble in ethylacetate and completely insoluble in ether, benzene, and light petroleum. (Found: C=54.6, 54.42; H=6.78, 6.73; Pb in the lead salt=32.4, 32.8 %.)

The filtrate after the removal of the lead compound described above, gave another precipitate with basic lead acetate. This second lead salt on decomposition with hydrogen sulphide, removal of the lead sulphide and complete evaporation of the mother liquor gave a dark red amorphous powder which was found to have properties similar to tannin colouring matters. Thus it dissolved in alkalies with a dark yellow colour from which the original compound was precipitated unchanged on treatment with acid. Concentrated sulphuric acid dissolves the substance with a dark brown colour and the solution chars on heating. It does not reduce Fehling's solution. Its

general properties and solubility are allied to the substance described above. (Found: C, 53.2, 53.44; H, 5.7, 5.5%.)

*Examination of the mother liquor from the above.*—The mother liquor from the above substance was treated with hydrogen sulphide and after the removal of lead as sulphide, the filtrate was evaporated when a large amount of crystalline matter was obtained. This was found to be entirely inorganic. The mother liquor from this contained only reducing sugars.

*Examination of the brown resin described before.*—The substance was freed from oily impurities by extraction with petroleum ether and was treated in alcoholic solution with alcoholic lead acetate. The voluminous precipitate thus obtained was washed with boiling alcohol and was decomposed in alcoholic suspension with hydrogen sulphide. The filtrate on evaporation gave a dark brown amorphous powder melting at 120-122° and having a slightly astringent taste and characteristic smell of the drug. It is soluble in alkalies with a yellow colour from which it is reprecipitated with acids. It is soluble in alcohol, acetone and pyridine, sparingly soluble in acetic acid, ethyl acetate and water and insoluble in benzene, chloroform, carbontetrachloride and petroleum ether. It gives a green coloration and precipitate with alcoholic ferric chloride. From the reactions it appears to be a phlobaphene. (Found: C, 57.9, 57.53; H, 7.16, 6.73%; Pb in the lead salt 36.16, 36.3%.)

The mother liquor from the above lead acetate precipitate was treated with hydrogen sulphide to remove the excess of lead and after removal of the lead sulphide, the alcoholic filtrate was evaporated, when a bright yellow amorphous powder was obtained melting at 74° and having an extremely bitter taste. This was separated into two portions by ethyl acetate, one being soluble in the solvent and the other insoluble. The ethyl acetate soluble portion was apparently the *Neriodorin* obtained by Greenish. It is a bright yellow amorphous powder melting at 86-87° and having an extremely bitter taste. Concentrated sulphuric acid dissolves this substance with a bright red colour and nitric acid with a yellow colour. It is very soluble in alcohol, acetone, acetic acid and pyridine, moderately in ethyl acetate and insoluble in water, benzene, chloroform and ether. It does not reduce Fehling's solution except on hydrolysis. It is slightly laevo rotatory,  $(\alpha)_D^{30} = -1.04$ . [Found: C=65.28, 64.94; H=7.43, 7.69%; M. W.—ebullioscopic in acetone—390, 394, 388.  $C_{22}H_{32}O_7$  requires C=64.7, H=7.8%, and M. W.=408.]

The hydrolysis of *neriodorin* was carried out by moderately strong hydrochloric acid under reflux in alcoholic solution. The aglucone thus obtained was a yellow amorphous powder melting at 68°, and was altogether tasteless. The sugar obtained by hydrolysis was identified to be glucose by means of the osazone.

The ethylacetate insoluble portion of the resin described above was purified by repeated precipitation from alcoholic solution and was obtained

as a bright yellow amorphous powder melting at 106-107°. This substance has been named *Neriodorein* according to Greenish. It dissolves in concentrated sulphuric acid with a brownish violet colour and in nitric acid with an orange colour. Its general properties and solubility are practically very similar to the compound described above, *i.e.*, to neriodorin. [Found: C=56.4, 56.57; H=7.3, 7.17%; M. W.—ebullioscopic in methyl alcohol—483, 499, 494.  $C_{23}H_{34}O_{11}$  requires C=56.79; H=7.02% and M. W.=486.]

The hydrolysis of neriodorein was carried on in the same way as in the case of neriodorin. The aglucone on repeated purification from alcohol was obtained as a yellowish white amorphous powder, melting at 70°. The substance is absolutely tasteless. The sugar was identified to be glucose.

In conclusion, one of the authors (G. P. P.) desires to express his indebtedness to the "Kanta Prasad Research Trust" of the Allahabad University for a scholarship which has enabled him to take part in this investigation.

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DIAGRAMMATIC REPRESENTATION OF THE ANALYSIS OF THE ALCOHOLIC EXTRACT FROM THE  
BARK OF *NERIUM ODORUM*

HOT ALCOHOLIC EXTRACT

Solid Wax separated on cooling; M. P. 97°C. (1.6%) Concentrated to 300 c.c.

Chlorophyll and other Waxy material separated out

Steam distilled

Distillate—Pt. Ether extract on evaporation to dryness—a few drops of essential Oil.

Residue extracted with Pt. Ether and evaporated to dryness—Very small quantity of Waxy material and a few drops of fixed Oil.

Distilled water added.

Filtrate (*Water Soluble Portion*)  
Aqueous solution of lead acetate added.

Resinous brown mass (*Water Insoluble portion*) Redissolved in alcohol and alcoholic solution of lead acetate added.

Pb. salt (1) Decomposed by  $H_2S$  in water suspension and the product isolated. *Amorphous Tannin* M. P. 240°C. (2%).

Filtrate, excess of lead removed by  $H_2S$   
Basic lead acetate solution added.

Lead salt (3) Decomposed by  $H_2S$  in alcoholic suspension and the product isolated. *Brown amorphous Phlobaphene*. M. P. 120-22°C. (35%).

Filtrate, excess of lead removed by  $H_2S$  and evaporated to dryness. *Bright yellow amorphous powder* Extracted with Ethyl acetate.

Basic lead salt (2) Decomposed by  $H_2S$  in water suspension and the product isolated. *Deep red amorphous colouring matter* does not melt up to 250°C.

Mother liquor, excess of Lead removed by  $H_2S$  as above and concentrated.

Residue, purified and the product isolated. *Neriodorein* (6%) M. P. 106-107°C.

Ethyl acetate extract, evaporated to dryness and the product isolated (5%) M. P. 86-87°C.

Inorganic Salts crystallised out.

Sticky, Syrupy mass, mostly reducing sugars.

# CYTOPLASMIC INCLUSIONS IN THE OOGENESIS OF PASSER DOMESTICUS

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## INTRODUCTION

Oogenesis of various kinds of animals occupies a distinctly significant place in the rank of current cytomorphic literature. The selection of bird as a type rests on its suitability as a material for investigation and the ease with which it can be obtained.

Sparrows were collected in the suburbs of Allahabad from October 1932 to February 1933 and the fixatives employed were those of Da Fano, Cajal, Ludford, Regaud, Regaud-Tupa, Zenker-Helly, F. W. A., Champy Kolatschev, Champy-Nassanov, and Bouin. Neutral red was prepared as directed by Bhattacharya in Bolles Lees' Vade mecum. This piece of research was carried on in the Zoological Laboratory, Allahabad University, under Prof. Bhattacharya to whom my cordial thanks are due for his constant guidance and help. The historical part has been omitted in this paper. It is exhaustively dealt with in the papers by Brambell (1925) and Das (1932).

## Observation

### *Golgi Apparatus*

In the youngest oocyte that can be obtained the Golgi apparatus appears in the form of a few discrete spherical granules seen prominently against the crystalline background of the cytoplasm (Fig. 1). At this stage the nucleus occupies the larger portion of the cell and the cytoplasm is of a uniform consistency with the few grains of the Golgi complex occupying a juxtannuclear area on the side farthest from the periphery. With the growth of the cell there occurs a definite reduction in the relative size of the nucleus, while the cytoplasm shows a corresponding increase in bulk. The Golgi bodies grow much larger in number and embrace the nucleus from all sides, thus exhibiting a decided tendency towards a perinuclear condition (Fig. 2). Ultimately the Golgi bodies completely encircle the nucleus though the greater



portion of the main mass remains confined to the original area (Fig. 3). The cytoplasm, however, fails to maintain its uniform consistency, the juxtanuclear cytoplasmic seat of the Golgi apparatus (D'Hollander's Volk Nucleus of Balbiani) being obviously of a much denser texture than the rest of the cytoplasm (Figs. 4 and 5). In the middle of this restricted area appears a clear space lodging two small granules (Fig. 4). The small dark grains scattered over the dense juxtanuclear cytoplasmic area (not shown in Fig. 4) are identified as the Golgi bodies while the clear space carrying the two granules as the centrosome lodging the diploid centrioles. This mass, later on, begins to break up in a peculiar fashion. The Golgi elements of the interior either completely disintegrate and disappear or migrate towards the periphery of the mass. The result of the process is the formation of a capsular structure with a clear central space and a sort of wall built up of Golgi bodies (Figs. 3 and 5). The clear cytoplasm of the interior is much denser and corresponds to the 'archoplasm' of other writers. This stage is of a short duration and does not always intervene as a definite step in the morphological changes the Golgi apparatus undergoes. When it does occur it is sometimes followed by a re-accumulation and further growth of the Golgi bodies. The mass ultimately breaks up and the Golgi bodies disperse through the cytoplasm till they are evenly distributed throughout. Later on, due to their outward migration, a cortical concentrated band of Golgi bodies becomes established. And this band persists even after the elements of the interior have disintegrated and become ultramicroscopic. Ultimately this band also disappears and no Golgi elements can be detected anywhere in the cell. The Golgi bodies are generally in the form of solid spherical grains and do not show the chromophilic and chromophobic parts.

### *Mitochondria*

Like Golgi apparatus, mitochondria also occur as a few discrete granules very close to the nuclear membrane at one pole in a young oocyte (Fig. 7). With the growth of the cell they increase in number but remain confined to this pole with the result that a concentrated mitochondrial mass gets formed on this side of the nucleus (Figs. 8 and 10). The granules are scattered over a dense cytoplasmic stratum which stands out in sharp relief against the background of the nearly clear cytoplasm (Fig. 9). Obviously this is 'archoplasm' as mentioned previously. The mitochondrial mass gets later transformed into a transitory capsular structure (Fig. 10) appearing almost as a counter-part of the similarly-shaped structure mentioned in connection with the Golgi apparatus. The mass ultimately disintegrates and the elements undergo rapid dispersal till they are evenly distributed over the entire cytoplasm. This is followed by a cortical concentration of the mitochondria brought about by the outward migration of the granules of the interior. Still later three distinct concentrated

bands of mitochondria become established—one perinuclear, the second middle and the third cortical (Fig. 11). The granules in the intervening regions are few and are drifting away. The granules become very fine but could be detected even in the biggest egg the writer could examine. The mitochondria are in the form of granules and rods, the granules being the more common.

### *Egg Membranes*

The earliest oocytes are almost naked the only covering being a poorly preserved extremely thin membrane (Fig. 1). There is no sign of any follicle cell as yet and no trace of any fibrous sheath. Very shortly, however, some cells can be perceived lying irregularly around the oocyte and later, by rapid multiplication, they form one continuous covering for the egg. Between the follicular layer and the periphery of the egg there is no intervening membrane of any description. Outside the follicular layer there is a fibrous sheath of connective tissue. In the early stages this band does not show any differentiation into sharply divided layers (Fig. 13) but during the later stages of oogenesis and especially at the time when the follicular epithelium becomes many-layered a differentiation is detected in the staining capacity of the thecae. The internal layer or the theca interna stains more intensely than the theca externa. The cells of the thecae are elongated and their nuclei have a similar shape. Their cytoplasmic inclusions—Golgi bodies and mitochondria—are fine granules. The Golgi bodies of the follicular epithelium occupy a position between the nucleus and the cell wall next to the periphery of the oocyte. At this stage the epithelium is a single-layered band completely enclosing the egg and no zona radiata has yet been formed. It is at this stage that the "infiltration" of the Golgi bodies occurs (Fig. 5). The Golgi elements lying at the periphery of the cell in the figures 3 & 5 have been transported from the follicular cells and do not belong to the egg proper. The infiltrating elements are in the form of spherical granules and not solid lumps.

Figure 6 represents a much later stage. Two definite layers of fibrous sheath—theca externa and theca interna—have become established. Zona radiata, though not outstandingly prominent, is a more or less distinct structure. The Golgi bodies are uniformly scattered but the follicular epithelium is still single-layered. The Golgi apparatus of the follicle cells is a patch of fine grains situated in a juxtanuclear position, facing towards the egg proper. And these fine grains are filtering down into the oocyte. Just beneath the zona radiata lying in the periphery of the egg is a band of Golgi bodies.

The single-layered condition of the follicular epithelium persists only for a brief interval. The cells undergo rapid multiplication and with the increase in the size of the egg proper grow considerable in number. The epithelium

becomes double-layered (Fig. 11) and eventually may become multi-layered. It is generally at this stage that the zona radiata is fully formed and is distinctly perceptible. During the formation of the additional layers of the follicular cells a process of differentiation sets in. Some of the cells stain much more deeply than the others and in between the normal lightly staining cells are also found non-cellular patches which have taken a deep homogeneous stain (Fig. 8). These exhibit apparently the extreme condition to which the dark cells are reduced. The lighter cells possess the normal cytoplasmic inclusions (Golgi bodies and mitochondria). Not so the dark cells. They do not appear to possess these inclusions and have fallen into a state of decay and degeneration. The nuclei of the cells contain a number of deeply staining nucleoli. They colour deep red with acid fuchsin and intense blue with haematoxyline.

The zona radiata does not show any structure and no follicular prolongation into the substance of the zona radiata could be made out. It does not exhibit any evidence of a differentiation into two well marked layers and persists as a single, homogeneous non-striated band.

The mitochondria do not infiltrate at any stage of the oogenesis, and are present in the follicle cells as fine grains. Like Golgi bodies they occupy the juxtannuclear position facing the egg periphery.

In close proximity to the thecae occur numerous luteal cells presenting a glandular appearance.

The follicle cells sometimes show abnormal activity. They multiply rapidly and invade the cytoplasm of the egg and eat away, as it were, the entire egg.

#### *Yolk-bodies*

An examination of the osmic-treated material clearly shows that only one kind of yolk is present—the fatty yolk. There is absolutely no trace of any albuminous yolk. In osmic-fixed preparation these bodies appear dense black and not pale-yellowish as is often, though not always, the case with albuminous yolk, and a short immersion in acid-free turpentine decolourizes them. They are never fixed by the current non-osmic fixatives and are extremely unstable. Even the xylol of Canada balsam dissolves them out in the mounted sections (Fig. 5). Staining with Sudan III gave the confirmatory reaction of fat and they got coloured with neutral red if left in that vital dye for a sufficiently long period, say 90 to 100 minutes. They never stain with acid fuchsin.

The centrifuge experiment throws them to the upper pole, these being the lightest inclusions. The centrifuge experiment also clearly demonstrates the absence of albuminous yolk. The empty vacuoles arranged on the cortex in non-osmic preparations are fatty yolk vacuoles which were not

preserved by the fixatives and were washed out in the subsequent process (Fig. 6).

The fatty yolk bodies arise in the juxtanuclear area and also on the cortex. It is usual to find them arranged on the periphery but in many cases they fill the interior as well.

The fatty yolk does not arise independently in the ground cytoplasm but in intimate association with the Golgi bodies which are mainly responsible for their formation. All gradations between the minute particles of the Golgi bodies and the swollen spheres of yolk are present (Fig. 12). The periphery is crowded with big and small spheres of fatty yolk with the Golgi bodies scattered in between them. Besides, on treating the sections with acid-free turpentine or immersing the slide for a long time in xylol yolk dissolves out but black crescents and granules are left bordering the empty vacuoles. (Fig. 13).

With the growth of the oocyte the fatty yolk bodies increase enormously in number and also swell up to attain greater dimensions. There is, however, distinctly perceptible a stage beyond which the fatty yolk bodies tend to become distinctly smaller though concomitantly more numerous.

An abundance of fatty material is found to be filling very young oocytes in certain cases. This fat is much more unstable than the ordinary Golgi yolk and in fixed preparations the cells containing them present a highly vacuolated appearance. The archoplasm stands out in strong contrast in such cells. This appears to be a case of fatty degeneration and not a normal process.

### *Intravital*

Examination of thin strands of ovary in salt solution under strong artificial light (1000 candle power) did not show the Golgi bodies. The younger cells were comparatively free from yolk globules and the cytoplasm appeared a clear crystalline expanse containing a few refringent granules. In very young oocytes a dense juxtanuclear area corresponding to the "Yolk Nucleus of Balbiani" could be picked out. The older cells were densely packed with fatty yolk spheres.

Treatment of fine pieces of ovary with neutral red brought out Parat's vacuome. In early cells these red granules were confined to the "Yolk Nucleus of Balbiani" area but in more advanced ones they were scattered throughout the cytoplasm (Fig. 14). These minute red granules were generally scattered individually but were also found in groups (Fig. 14). Occasionally some of them ran into each other to form a single big structure

(Fig. 14). The "Vacuome" of the follicle cells are likewise in the form of fine red granules scattered throughout the follicle cells. It takes nearly an hour before the vacuome are distinctly seen, though half an hour may suffice in some cases. After the vacuome have clearly come out a little quantity of 1% osmic acid is run into from one side and drained from the other. The Golgi bodies come out as homogeneous grains and crescents. In the advanced oocytes they are homogeneously distributed (Fig. 14) but in young eggs they are confined to the juxtannuclear area. The Golgi bodies of the follicular epithelial cells are minute particles mostly gathered in a patch near the nucleus.

A simultaneous application of neutral red and janus green B met a failure. Pieces of ovary separately treated with janus green B showed mitochondrial patches with remarkable clearness. Mitochondria were in the form of rods and granules situated in a patch on a denser substratum of cytoplasm. These patches were regularly arranged concentrically round the nucleus (Fig. 15) and the arrangement continued in sufficiently advanced oocytes.

The mitochondria of the follicular cells were invariably found in the form of a prominent juxtannuclear patch facing the periphery of the egg.

Fatty yolk spherules get blackened on prolonged treatment of the tissue with osmic acid. But for the confirmation of the presence of fat Sudan III test is more satisfactory. It was used on fresh and formalin-treated material and in both cases showed the usual red reaction.

## Discussion

### *Golgi Apparatus*

By a majority of prominent cytologists the Golgi body is treated as a permanent structure independent of any canalicular or vacuolar apparatus and possessing a fundamental chemical and structural basis in spite of the diversity of form and function it may assume in different varieties of cells. It is credited with the power of growth, assimilation, and fission and is considered a cytoplasmic inclusion of universal occurrence. It is not considered a product of metabolism.

Still there is no undisturbed unanimity. Walker and Allen (1924) openly condemned Golgi apparatus as an artefact produced by the action of fixatives on the cytoplasmic colloids. Benseley and Helen (1929) showed that osmication involves no chemical reaction but is simply a process of reduction, dispersal and adsorption. Strangeways and Canti (1927) did not find Golgi bodies in their dark ground experiment on tissue culture cells. Chambers (1931) in the course of his micro-dissection found no area of resistance in the cell.

The Golgi apparatus has, however, been seen intravital. Gatenby easily observed them in male germ cells of *Cavia* and *Abraxas*, by applying neutral red. Gatenby, Rau, and Brambell were able to produce microphotographs of the Golgi bodies of living male germ cells. Bhattacharya and Das (1929) demonstrated it by vital methods in the oocytes of pigeon. Nath saw the apparatus intravital in the eggs of earthworm and frog. Das (1932) observed it in the living unstained oocytes of pigeon.

The observation of the present writer is in conformity with those of the latter group. The Golgi apparatus is easily seen on treating the fresh ovary with 1% osmic acid for a short time and though the method is not strictly intravital, it does not involve any danger of artefact. (Strangeways and Canti 1927.)

The morphology of the Golgi apparatus admits of extensive variation. Cajal considered it a system of canals with a limiting membrane enclosing lipoids. Hirschler (1913, 1914, 1916, 1918) advanced the theory that the Golgi apparatus is of a duplex character and essentially lamellar in construction. An existent representative of Hirschler "Apparatinhalt" was found by Bowen in the chromophobe of the apparatus. Kunze 1921, Brambell 1923, Brambell and Gatenby 1923 showed that in the nerve cells of *Helix* the Golgi body consists of curved rods or ring-shaped dictyosome. Harvey showed it to be scally in form but Nath in a series of papers on oogenesis declared that the only form of the apparatus is vesicular with chromophilic cortex and chromophobic interiors. Bhattacharya described many forms in the tortoise,—rodlets, platelets, crescents, beaded strings, etc. (1925).

In the material under investigation the Golgi bodies were mostly in the forms of spherules, rods and crescents. It does not, however, seem necessary to reduce the morphology of the Golgi apparatus to a standard form.

The functional significance of the apparatus in oocyte remains obscure. In many cases it has been shown to form fatty yolk. But beyond this little more is known. In male germ cells it forms the "acrosomes" (Bowen, Gatenby and others) and in glands it is held responsible for the formation of the secretory material. Its function thus varies with the nature of the cell in which it is lodged. In female germ cells its only function known so far is to supply nutriment to the developing ovum. In the eggs of *Passer domesticus* the Golgi apparatus is concerned with the same function.

#### *Mitochondria*

Mitochondria have been seen intravital in the eggs of a number of animals by workers in this laboratory. The dark ground experiments of tissue culture conducted by Strangeways and Canti (1927) conclusively showed their presence in living tissues and Chamber's report (1931) is equally

confirmatory. In the present work mitochondria were seen by intravital methods very clearly.

In the eggs mitochondria are generally present in the form of granules and rods. In a few cases the filamentous forms have also been described; (Hibbard in *Discoglossus*, Hibbard and Parat in *Pygostens* and *Perca*, Harvey in *Lumbricus*, King in *Peripatus*, Bulliard in *Emy lutaria*, Gatenby in *Apanteles* and Bhattacharya and collaborators in a number of animals from our laboratory).

The present writer finds only granular and rod-like mitochondria in the eggs of *Passer domesticus*.

Very early oocytes of the material under investigation were entirely free from mitochondria. Gardiner in *Limulus* and Harvey in *Carcinus* met a similar failure and a number of workers from this laboratory have reported similar cases. The continuation of mitochondria from the undifferentiated germ cells to the segmenting ovum is, however, a well-established belief among the cytologists and there is a wealth of evidence to support it. It is possible that at very early stages they are in ultra-microscopic condition and hence escape detection.

Mitochondria play absolutely no part in the process of vitellogenesis of the oocytes of *Passer*. This is in sharp disagreement with the reports of Brambell on the oogenesis of domestic fowl and of Das on pigeon who ascribe the formation of proteid yolk to mitochondria.

Van Durme's account of mitochondria contains a description of three zones into which the mitochondria get concentrated during the early stages of dispersal. These three zones are prominently seen in the eggs of sparrow but they never give rise to proteid yolk. Brambell does not record these layers and Das mentions only an ultimate cortical concentration. This cortical band is an intermediate stage in the oogenesis of *Passer domesticus*.

#### *Egg-Membranes*

The mature egg of this bird is enveloped by theca externa, theca interna, follicular epithelium and zona radiata. I have not met the cortical fibrillated layer situated between the periphery of the cell and the zona pellucida as mentioned severally by Gatenby and Bhattacharya. And the zona radiata is a single and not a permanently double-layered structure. Das (1932) thinks this fibrillated layer to be the vacuolated part of the cortex. There is no such vacuolated area in the cortex of the oocytes of the *Passer*, the only vacuoles being those of the fatty yolk. What Gatenby and Bhattacharya described as fibrillated layer is probably a separate structure not found in birds.

The zona radiata has been described as marked by definite radiations and traversed by hollow strands of cytoplasmic material of the follicle cell in fishes, reptiles, mammals and birds (Loyez, Van-der Stricht, Champy, Thing,

etc.). The zona radiata of Passer is unstriated and does not show any prolongation of follicular substance.

Hall, Brambell, Mertens and Das described two kinds of cells in the follicular epithelium of the oocytes of birds and fishes—clear and dark cells. My observation is in agreement with those of these workers. The differentiation of the dark cells from the light normal cells is brought about by a process essentially degenerative in character, the non-cellular dark patches being the extreme stage. These dark patches seldom or never extend across the full breadth of the follicular layer and there is little reason to ascribe a mechanical function to these, as Brambell has done. Further, it is significant to note that nearly all the follicular cells in cases of abnormal activity are like dark cells—dark and devoid of the usual cellular inclusions.

The nutrition of the oocyte demands an inflow of nourishing substance through the enveloping layers and the actual occurrence of such a process has been known for long (Loyez 1905, Waldayer 1870). The nature of granules filtering down into the cytoplasm of the egg has been only recently investigated (Bhattacharya and Brambell). The infiltration in the oogenesis of this bird occurs both before and after the formation of the zona radiata. Das (1932) came to a similar conclusion, but Brambell has recorded only one stage. The significance of the intrusion of the Golgi bodies becomes apparent on the assumption of their nutritive function.

#### *Vitellogenesis*

For a bird the absence of albuminous yolk is rather an exceptional fact. Van Durme (1914), Brambell (1925) and Das (1932) all found it and traced its formation to the mitochondria. Mitochondria have been held responsible for albuminous yolk formation by various other workers in different animals (Hirschler, Bhattacharya, Nath, Lams and Doorme, and Bhattacharya and collaborators from this laboratory). Nucleolar extrusions have been ascribed the same function in many cases (Gatenby, Nath, King, Gresson and others).

Weiner, Harvey and Steopoe derived albuminous yolk from Golgi bodies, while Nath, Parat and Hibbard and Parat declared "vacuome" to be the source in some cases. It is difficult to accept the former interpretation as of late the Golgi apparatus is being considered the main source of fatty yolk formation (Gatenby, Ludford, Brambell, Nath, King, Bhattacharya, Das and others). The observation of the present writer is in agreement with the interpretations of the latter group.

#### *Vacuome*

Parat's vacuome hypothesis considers the classical Golgi apparatus an artefact created by metallic precipitation in and around a system of neutral red-stainable vacuoles which are the real pre-existent organelles of the cell.



Benseley first described a canalicular apparatus in the cell and Guilliermond and Mangelot later emphasized the canalicular nature of the Golgi apparatus in plant cells. Guilliermond extended the hypothesis to animal cells. But a complete vacuolar hypothesis was put forth by Parat and Painleve (1924).

Parat contended that under the influence of cytological fixatives the vacuoles get disorganised, run and coalesce together to form the typical Golgi network. His work centred mainly round secretory materials. Krjukowa (1929), Beams and Goldsmith (1930) repeated Parat's work on salivary gland cells of chironomus larva and reported that they did find the typical crescentic Golgi bodies which Parat had overlooked.

Parat, however, had to explain the dictyosomes stuck over the archoplasm in many germ cells (Gatenby, Bowen, etc.) Parat called them "*chondriome actiff*" or modified mitochondria while the vacuoles they enclosed were termed the "vacuome." Avel, Gatenby, Bowen, Pollister and others definitely rejected this view. This "Lepidosome" theory never attracted any enthusiastic support.

Parat's vacuome theory was supported by workers on protozoan Cytology (Joyet and Lavergne, Hall, Hirschler, Volkonsky, Lwoff and Lwoff, Cowdry and Scott and many others). Covell and Scott as a result of their neutral red experiments on spinal Ganglion cells came to the same conclusion. Beams (1931) emphatically contradicted this and showed that the interpretation of Covell and Scott was erroneous.

Neutral red itself does not seem to be a very specific dye. Vera Koehring (1929) coloured several system of Protozoa with it which cannot be homologized with Parat's vacuome. Then the work of Chlopin (1927, 1928), Weiner ('30) shows that the neutral red, though the least toxic vital stain, is responsible for creating artefacts and thus causes misinterpretation. In his latest paper on chironomus larva salivary gland cells Gatenby showed that neutral red created artificial spaces that were mistaken for pre-existent structures (Vacuome) and that the independently occurring Golgi bodies were real but separate bodies.

Moreover, the Golgi apparatus and vacuome have been seen simultaneously by many workers (Tretjakoff, Grabowski, Rumjantzew, Beams, Beams and King, Bhattacharya and Das, and Nath). The separate roll of the two structures in spermatogenesis was worked out by Voinova, Payne, Pollister, Hirschler, Monne and Gatenby.

In the oocytes of *Passer domesticus* the two structures are completely independent and have been simultaneously demonstrated. There is no reason to suppose the vacuome to be a secretory product of the Golgi body and at the same time it is difficult to treat "Vacuome" as a mere artefact. They cannot be brought out in dead cells and did not collapse on subsequent osmication.

*Nucleolar Extrusion*

There is no sign of nucleolar extrusion in this animal.

## SUMMARY

In the oogenesis of *Passer domesticus*

1. The Golgi bodies appear in the form of a few granules in young oocytes, grow in number, form the "Yolk-Nucleus of Balbiani" with a clear central space, spread out, and get arranged on the cortex in a concentrated band. They ultimately disappear.
2. Centrosomes and centrioles have been demonstrated in the Yolk Nucleus of Balbiani area.
3. Golgi bodies give rise to fatty yolk.
4. Mitochondria appear in early oocytes as a few granules which increase in number, form Yolk Nucleus of Balbiani, then spread out and form three concentrated layers.
5. There is no albuminous yolk.
6. Vacuome and Golgi bodies have been shown to be independent structures and are shown intravital.
7. Mitochondria have been demonstrated by intravital methods.
8. There is no sign of nucleolar extrusions.

## EXPLANATION OF LETTERING

G. B.	...	Golgi Bodies.	Mi. P.	...	Mitochondrial Patch.
Mi.	...	Mitochondria.	Va.	...	Vacuome.
Y.N.B.	...	Yolk Nucleus of Balbiani.	G. Gr.	...	Golgi granules.
F.G.B.	...	follicular Golgi bodies.	G. Cr.	...	Golgi crescents.
F.C.	...	Follicle Cells.	F.Y.B.	...	Fatty Yolk body.
F.N.	...	Follicular Nucleus.	C.S.	...	Central space.
F. Nu.	...	Follicular Nucleolus.			
N.	...	(Oocyte) Nucleus.	B.V.	...	Blood Vessel.
L.M.C.	...	Layer of Mitochondrial Concentration.	F.V.	...	Fatty vacuoles.
Inf.	...	Infiltration.	V.	...	vacuoles.
Th. in.	...	theca interna.	L.C.	...	Light Cell.
Th. ex.	...	theca externa.	D.C.	...	Dark Cell.
Z.R.	...	Zona Radiata.	N.C.P.	...	Non-cellular Patch.

## EXPLANATION OF PLATES

Fig. 1. Early oocyte showing a few juxtannuclear Golgi granules. Da Fano, toned.

Fig. 2. More advanced oocyte. Golgi bodies have grown in number and are spreading on sides Da Fano, toned.

Fig. 3. Golgi bodies have surrounded the nucleus. Yolk Nucleus of Balbiani with a clear central space. Infiltrated Golgi bodies are on the periphery. Da Fano toned.

Fig. 4. Yolk Nucleus of Balbiani with centrosomes lodging centrioles. Da Fano toned and stained with Iron-alum haematoxyline.

Fig. 5. Yolk Nucleus of Balbiani with the central space. Golgi bodies are spreading out and fatty vacuoles are present. Infiltrated Golgi bodies are on the periphery. Ludford bleached.

Fig. 6. Infiltration of Golgi bodies. Zona radiata is forming and thecae are two-layered (Cajal).

Fig. 7. Young oocyte showing a few mitochondrial granules. F.W.A. Acid Fuchsin.

Fig. 8. Yolk Nucleus of Balbiani. F. W. A. Acid Fuchsin.

Fig. 9. More advanced oocyte. Mitochondria are present in two patches and are spreading out. F.W.A. Acid Fuchsin.

Fig. 10. Microphotograph of an advanced oocyte showing a juxtannuclear patch of mitochondria with the central space. F W. A. Iron-alum Haematoxyline.

Fig. 11. Mature oocyte. Mitochondria are arranged in three concentrated layers. Zona radiata is formed; dark cells and non-cellular patches are formed. Follicular epithelium is two-layered. Thecae have differentiated into two layers.

Fig. 12. Part of an old oocyte showing the formation of fatty yolk bodies. Theca externa, theca interna, and blood vessels are shown. (Ludford.)

Fig. 13. Showing Golgi bodies left after dissolving out fat. (Ludford.)

Fig. 14. Part of an old oocyte showing vacuomes and Golgi bodies. Neutral red and Osmic.

Fig. 15. A young oocyte showing concentric mitochondrial patches. Janus Green B.

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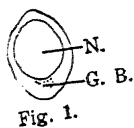


Fig. 1.

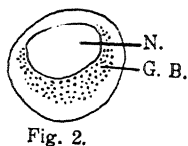


Fig. 2.

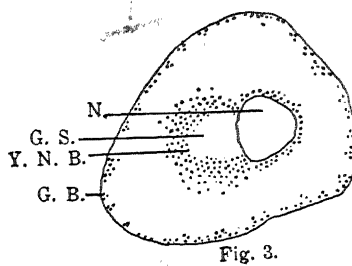


Fig. 3.

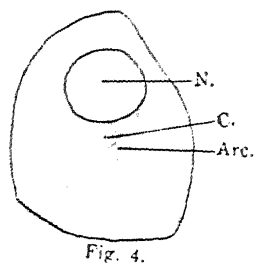


Fig. 4.

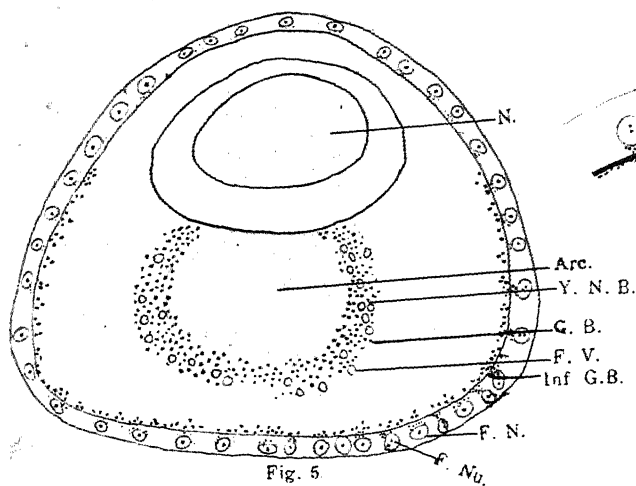


Fig. 5

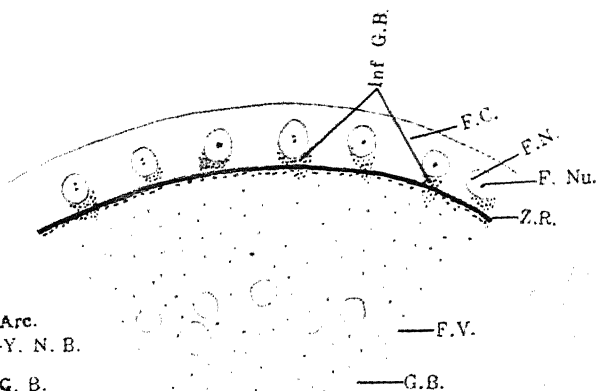


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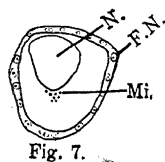


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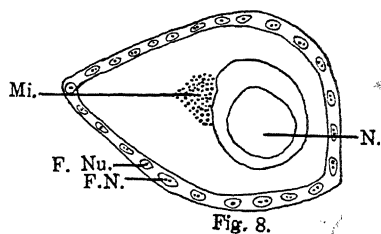


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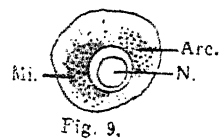


Fig. 9.

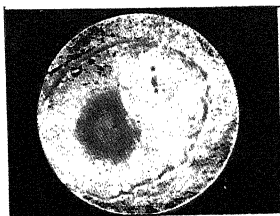


Fig. 10

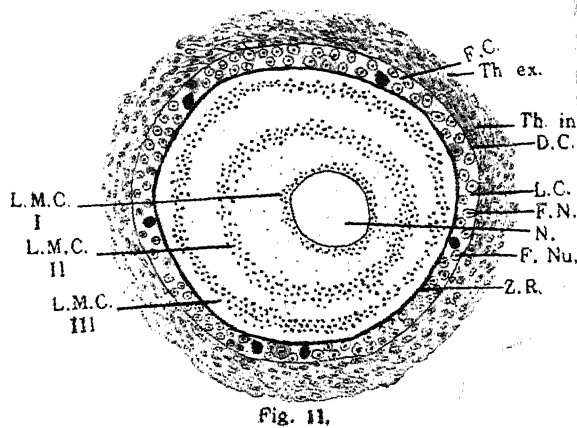


Fig. 11.



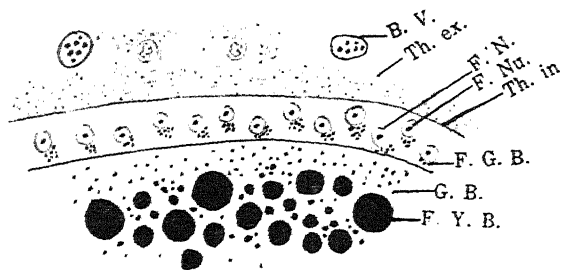


Fig. 12.

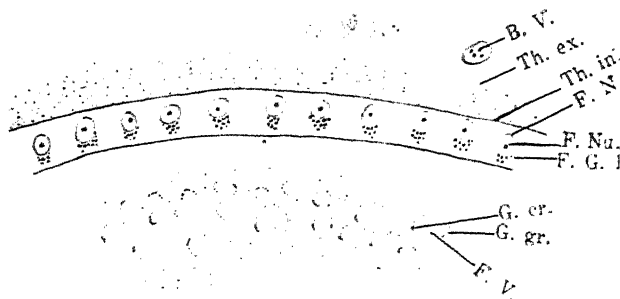


Fig. 13.

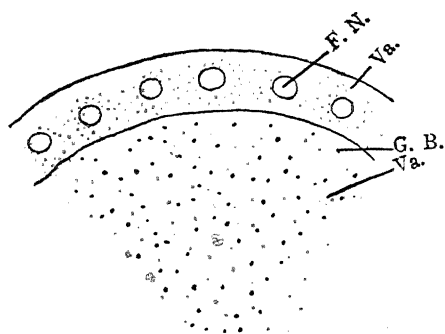


Fig. 14.

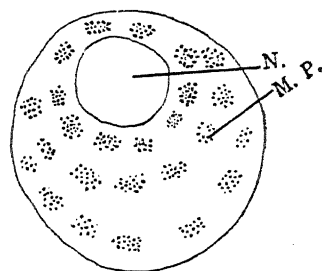
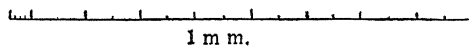


Fig. 15.



1 m m.  
Fig. 1,2,3,4,5,6,7,8,12,13,14,15.



5 m m.  
Fig. 9,11.





# ON THE ABSORPTION SPECTRA OF THE HALIDES OF ELEMENTS OF THE FIFTH GROUP

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This paper is an extension of my previous work<sup>1</sup> on the absorption spectra of some of the lower chlorides of the elements of this group. The substance investigated are the bromides and iodides. I have also examined antimony penta-chloride to see if there was any correspondence between the results obtained in the case of antimony trichloride previously examined and those obtained here.

As was said in the previous paper<sup>1</sup> these salts can be regarded as saturated compounds. As an illustration we may consider  $\text{PI}_3$ . Its constitution may be given by  $\text{P}^{+3} 3\bar{\text{I}}$ . Each  $\text{I}^-$  —ion is diamagnetic and  $\text{P}^{+3}$  has the constitution  $1s^2 2s^2 2p^6 3s^2$ ; hence  $\text{P}^{+3}$  is also diamagnetic. The case of bromide is similar and the same is the case with the halides of other elements of this group. All these substances should show continuous absorption.

Their atomic heats of formation are calculated as in the previous paper. They are calculated by the help of the following formula which is based on the Born cycle. In the case trihalides the atomic heat of formation in kcals is

$$R = Q + L_M + \frac{3}{2} D_{\text{Ha}_2} - L_{\text{MHa}_3}$$

where  $Q$  = heat of formation of the salt in kcals per mole.

$L_M$  = heat of vaporisation of the metal from the condensed state to the atomic state in kcals per mole.

$D_{Ha_2}$  = heat of dissociation of the halogen (Ha) in kcals per mole.

$L_{MHa_3}$  = heat of vaporisation of the salt in kcals per mole.

$L_M$  is in its turn calculated by the help of relation

$$L_M = \frac{L + D_1 + 2D_2}{4}$$

where  $L$  = heat of vaporisation of the metal from the condensed state to a vapour of tetra-atomic molecules.

$D_1$  = heat of dissociation of a tetra-atomic molecule into diatomic ones in kcals per mole.

$D_2$  = heat of dissociation of the diatomic molecule into monatomic ones.

As an example we take the case of  $AsI_3$ . Here  $Q = 28.8$  kcals;  $S = 26.5$  kcals;  $\frac{3}{2} D_{I_2} = 53.4$  kcals and  $L_{AsI_3} = 19.2$  kcals; whence we get the value for  $R$  to be equal to 89.5 kcals.

All the necessary data have been taken from Landolt and Bornstein's tables and from Mellor's Treatise on Inorganic and Physical Chemistry. In the case of certain compounds, however, the value for latent heat of vaporisation of the compounds is not given as such. In that case we obtain this value from the data for vapour pressures of the substance at various temperatures. The formula employed for getting this result is

$$L \text{ in cal.} = \frac{T_1 T_2}{T_2 - T_1} \log_e P_2 / P_1$$

where the pressures  $P_1$  and  $P_2$  correspond to temperatures  $T_1$  and  $T_2$  respectively given in absolute scale.

#### EXPERIMENTAL PROCEDURE AND RESULTS

The experimental procedure was similar to that described in my previous paper.<sup>1</sup> In certain cases the absorption began in the quartz region, whilst in others it began in the glass region. In the former case the source of radiation was a hydrogen tube run by a transformer with a current density of 100 mA. The spectrograph was a quartz one of  $E_3$  type. The plates used were process plates. In the other case the source of radiation was a pointolite lamp, the spectrograph was a glass one of the constant deviation type and the plates were Agfa panchromatic.

In the case of antimony pentachloride the substance was a liquid and it was kept in a side bulb which was maintained at the temperature of boiling

water. According to tables the saturated vapour pressure at this temperature is 63 mm. of mercury, while the pressure recorded in the manometer attached to the tube is 60 mm. The absorption tube was then heated successively to the temperatures mentioned in the horizontal row of table 2. Microphotographs of these plates were taken at the laboratory of the Muslim University, Aligarh, by Dr. Asundi and the beginning of the long wavelength limit of absorption could be easily inferred from these records. It was found that at the lower temperatures, the rise of the absorption curve was gradual and became more so when the temperature was increased. The long wavelength limit was found to be continuously shifting from the value  $\lambda$  3500 Å at 100°C. to  $\lambda$  4100 Å at 340°C. The experiment, therefore, shows that the long wavelength limit shifts with the temperature. To see if there was any retransmission, the furnace was kept at room temperature and the pressure of the vapour in the absorbing column was varied from 1 mm. to 76 mm. of mercury by gradual steps. It was found that there was no retransmission.

The other substances were solids. For the sake of convenience, a little of solid was placed in each case inside the absorption vessel and heated to the temperatures mentioned in the top row of table No. 2. Both the pressure and the temperature of the absorbing column were thereby varied. The long wavelength limit was determined in the same way as before. Generally at lower temperatures, the absorption curve was found to be gradual and it became sharp as the temperature was raised. For  $\text{SbBr}_3$  the effect was more marked.

All these substances were examined to see if there were retransmission in any of them. For that purpose the temperatures to which they were heated was varied from the room temperature to a maximum of 340°C. by very gradual steps. This ensured that the pressure of the absorbing column was changed from very low values to higher ones very gradually.

In all these cases with the exception of  $\text{AsI}_3$  and  $\text{SbBr}_3$ , no other change except the shift in the long wavelength limit of absorption could be noticed with the changes in the conditions of temperature and pressure. The long wavelength shift was, in each case, towards the red end of the spectrum.

In the case of  $\text{AsI}_3$  and  $\text{SbBr}_3$  there was, in each of them, a retransmission of light following a continuous absorption which in its turn terminated in another continuous absorption. Thus there were two long wavelength absorption limits in these two cases corresponding to the two continuous absorptions separated from one another by the small patch of retransmission. The retransmissions appear at a comparatively high temperature, which shows that a comparatively large pressure of the absorbing column is required to make those retransmissions appear. These results are given in table 3. It was found that as the temperature increased the retransmitted patch

contracted from both sides. A similar phenomenon was observed in the case of tinhalides.<sup>4</sup>

The microphotograms of the various absorption spectra are given in figures 1 to 6. For economy of space all the microphotograms for one substance taken under various conditions are compressed in one figure. The microphotograms have been taken along the spectra. The microphotogram is (A) of the continuous spectrum from the hydrogen tube, (B) of the continuous spectrum from the pointolite lamp, (1) the absorption spectrum of the vapour of the substance at 50°C., (2) the absorption spectrum at 100°C., (3) the absorption spectrum at 300°C., and (6) the absorption spectrum at 340°C.

Table 1

Substance	Long wavelength limit in $\frac{1}{\lambda}$ "v <sub>m</sub> "	$Q_m = \frac{Nh\nu_m}{J}$ in kcal	Heat of reaction required to convert solid element into monatomic vapour in kcal per gm. atom "L <sub>m</sub> "	Heat of formation of the salt in kcal per mole "Q"	Heat of dissociation of the halogen molecule into atoms in kcal per mole "D"	Heat of vaporisation of the salt in kcal per mole "L"	$\frac{R}{3}$ in kcal for trihalides and $\frac{R}{5}$ in kcal for pentahalides	$Q_m - \left[ \frac{R}{3} \text{ or } \frac{R}{5} \right]$ in kcal
Antimony pentachloride	4130	68.9	[27.6]	104.8	56.87	11.05	52.7	16.2
Arsenic tribromide	3248	87.6	26.5	59.1	45.22	[10]	[47]	47.6
Antimony tribromide	5450	52.2	[27.6]	76.9	45.22	3.5	56.2	-4.0
Phosphorus triiodide	3147	87.4	34.5	26.7	35.6	[12]	[34]	53.4
Arsenic triiodide	5616	50.6	26.5	28.8	35.6	19.2	29.8	20.8

N. B.—1. The values enclosed in brackets are uncertain because of the interpolation referred to in my previous paper.

2. v<sub>m</sub> corresponds to that long wavelength limit of absorption which is nearest to the red in those cases where there are more than one absorption limits.

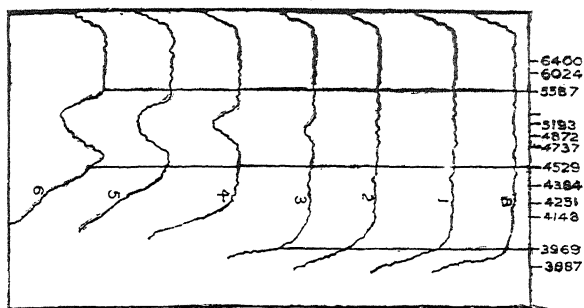


Fig. 1

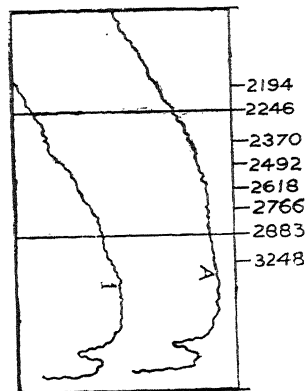


Fig. 2

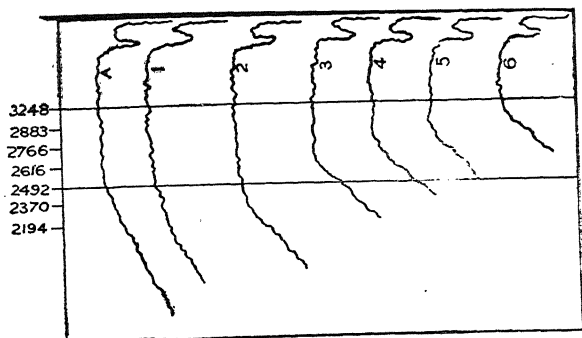


Fig. 3

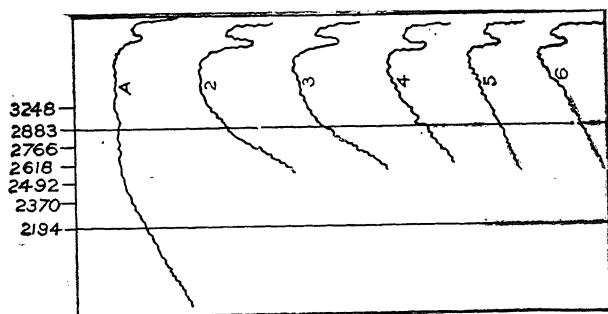


Fig. 4

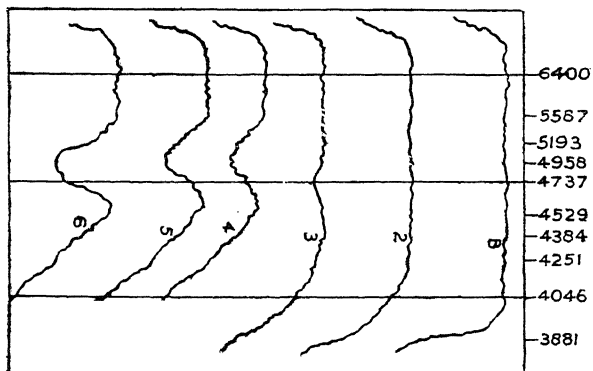


Fig. 5

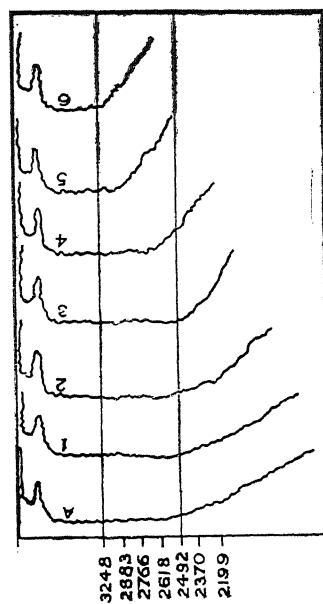


Fig. 6



Table 2

*Long wavelength limit of absorption of the vapours of the various substances at different temperatures in Angstrom units.*

Substance	Temperature in °C					
	50	100	180	250	300	340
Antimony pentachloride	...	3500	3670	3820	3965	4100
Arsenic tribromide ...	2100	2390	2595	2815	3020	3240
Antimony tribromide ..	3200	4110	4920	5444	5443	5450
Phosphorus triiodide ...	2095	2280	2485	2700	2920	3120
Arsenic triiodide ...	4002	4100	5070	5300	5465	5600

Table 3

Substance	First long wavelength limit of absorption "ν <sub>m<sub>1</sub></sub> "		Second long wavelength limit of absorption "ν <sub>m<sub>2</sub></sub> "		ν <sub>m<sub>2</sub></sub> - ν <sub>m<sub>1</sub></sub> cms. - <sup>1</sup>	Difference between the energies of the first two metastable states of the corresponding halogen (2P <sub>1/2</sub> - 2P <sub>3/2</sub> ) cms. - <sup>1</sup> .
	Å	cms. - <sup>1</sup>	Å	cms. - <sup>1</sup>		
Antimony tri-bromide	5260	19011	4670	21413	2402	7600
Arsenic tri-iodide	5440	18349	4600	21739	3390	3700

## DISCUSSION

Saha and Datta's hypothesis<sup>2</sup> says that the long wavelength limit of the absorption spectrum of the vapour of a substance which can be represented by MX<sub>n</sub> is given by  $\frac{R}{n}$  where R is its atomic heat of formation. We find, however, that the energy of optical dissociation is in each case different from one-third of the thermochemical value in the case of trihalides and one-fifth for pentahalides. As found in the chlorides<sup>1</sup> of this group the value is in excess to the thermochemical one. But this excess is not general. In the case of



antimony tribromide, for instance, the energy of optical dissociation is 52.2 kcal, whereas one-third of the thermochemical energy is 56.2 kcal. The thermochemical value is, however, not quite reliable. Its calculation involved the use of the value of the heat of vaporisation of antimony from the condensed state into monatomic vapour. This was obtained only by interpolation, on which much reliance cannot be placed. This deviation from the general behaviour may, therefore, be only fortuitous.

In the cases of  $\text{AsBr}_3$  and  $\text{PI}_3$  the atomic heats of formation ( $R$ ) are  $(153.4 - L_{\text{AsBr}_3})$  and  $(114.6 - L_{\text{PI}_3})$  kcal respectively. The melting points of  $\text{AsCl}_3$ ,  $\text{AsBr}_3$  and  $\text{AsI}_3$  are  $-13^\circ\text{C}$ ,  $31^\circ\text{C}$ . and  $146^\circ\text{C}$ . respectively and the heats of vaporisation of  $\text{AsCl}_3$  and  $\text{AsI}_3$  are 6.7 and 19.2 kcal respectively. From this it can be seen that the latent heat of sublimation of  $\text{AsBr}_3$  will be approximately 10 kcal and  $\frac{R}{3}$  will, therefore, be approximately equal to 47 kcal.

Similarly, the value of  $\frac{R}{3}$  in the case of  $\text{PI}_3$  is approximately equal to 34 kcal. The values of the energy of optical dissociation of these salts are equal to 87.6 and 87.4 kcal respectively. The difference between the optical and thermochemical values is very large. In the case of  $\text{AsBr}_3$  the optical value roughly equals  $\frac{2}{3}R$ . If this equalisation be considered and Saha and Datta's hypothesis<sup>2</sup> be taken to be true, we should consider it to be a case in which two bromine atoms have been knocked off simultaneously by the incoming radiation. This explanation appears to be improbable because the dislodging of one bromine atom from  $\text{AsBr}_3$ , whose probability is greater and which should manifest itself by another absorption did not take place. In the case of  $\text{PI}_3$  things were just the same. Here the value of the energy of optical dissociation was a good deal more than  $\frac{2}{3}R$  and as in the previous case no other absorption could be traced. This anomaly cannot, therefore, be attributed to the knocking off simultaneously of two halogen atoms from the molecule by the incident radiation.

It was found, in addition, that there are some compounds for which the long wavelength limit of absorption varies as we increase the temperature of the substance by fairly wide degrees. There are others for which the variation is very little. In the case of  $\text{SbBr}_3$ , for instance, after the substance had been heated to  $250^\circ\text{C}$ . the long wavelength limit of absorption does not further shift towards the red with greater increase in its temperature. In the case of others the shift towards the red takes place regularly as the temperature goes on increasing. The shifts are not alike in all the cases. In the case of  $\text{SbCl}_5$  and  $\text{AsI}_3$ , for example, the shifts are large at lower temperatures, but go on reducing as the temperature increases. In the cases of  $\text{AsBr}_3$  and  $\text{PI}_3$  they are equally large all over.

It is to be noted that in the case of  $\text{SbBr}_3$ , where there is no shift above about  $250^\circ\text{C}$ ., the values of the energy of optical dissociation and of  $\frac{R}{n}$  are nearly equal. In the case of  $\text{SbCl}_5$  and  $\text{AsI}_3$ , where the shifts go on reducing with the increase in temperature, the difference between the energy of optical dissociation and  $\frac{R}{n}$  is small, as compared to  $\frac{R}{n}$ . In the case of  $\text{AsBr}_3$  and  $\text{PI}_3$ , where the shifts remain the same all over the range of temperature, the difference between the two values is large as compared to the values of  $\frac{R}{3}$ .

These facts are very significant. Unfortunately, in these compounds heating brings about both a change in the temperature of the gas and a change in the pressure of the absorbing column. Experiments with hydrogen halides are being conducted where this ambiguity between the temperature and pressure effect could be eliminated at will. They can still be explained as follows. The transition from the stable electronic level to a higher unstable one takes place in the three cases as represented by Franck Condon diagrams<sup>3</sup> in the figures (a), (b) and (c).

In case (a) the upper level is disposed as shown in the figure. When the transition takes place from the lowest vibrational level of the stable electronic state it goes up to the higher state at a point where it is not horizontal. With the increase in the vibrational level in the lower state, brought about by an increase in temperature, there is both a horizontal and a vertical shift in the position of the point of maximum transition probability on the lower curve. The vibrational energy

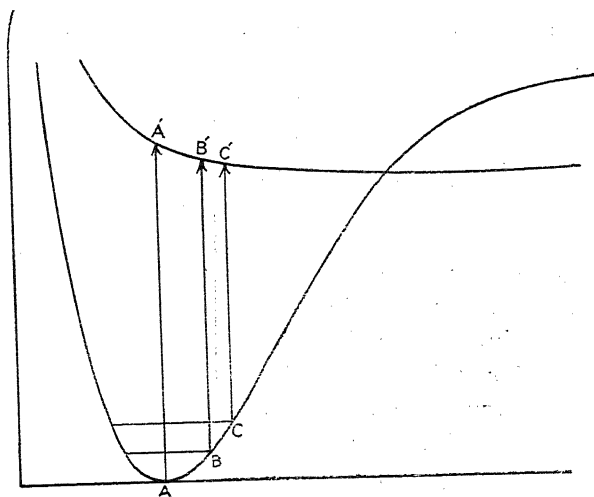


Fig. (a)

being small and the curve itself being not very much steep there, the vertical shift is very small as compared to the horizontal one. But with a horizontal shift in the position of maximum transition probability in the lower curve, the vertical distance as represented by the arrow get considerably reduced because of the slope of the curve. This vertical distance represents the energy of the beginning of absorption. This slope of the upper curve goes on reducing as we

go further to the right. Therefore on a further horizontal shift in the position of maximum transition probability brought about by an increase in the temperature of the substance we go to points having smaller slope on the upper curve and the vertical distance represented by arrows does not change so much. On the upper curve such points with a small slope are only very slightly above the horizontal line asymptotic to it. Since the height of this

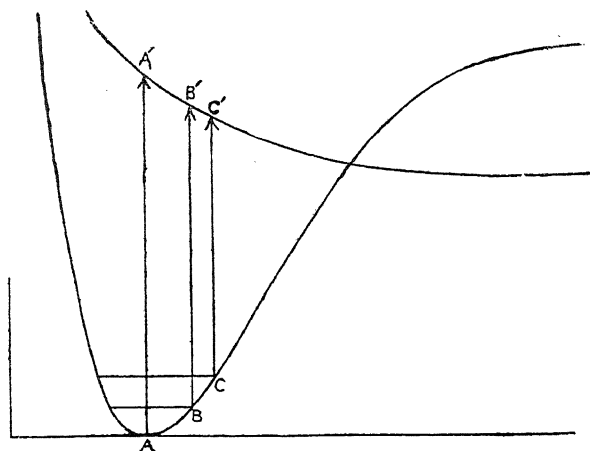


Fig. (b)

asymptote above the lowest vibration level of the lower state gives the heat of dissociation of the salt the value obtained optically is only very slightly greater than the heat of dissociation or it may be equal to or even a little less than the thermochemical value.

In case (b) the upper curve has got a greater slope at all the points to which the transition takes place at various temperatures used in the experiment. The horizontal asymptote is in this case below the most extreme right of these points. The heat of dissociation obtained optically is, therefore, greater than  $\frac{R}{3}$ .

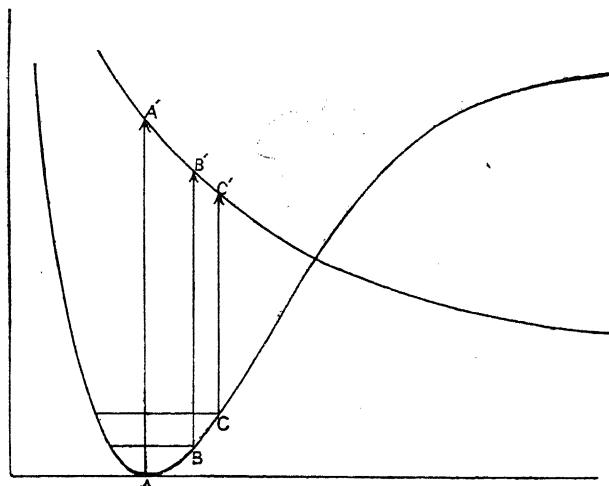


Fig. (c)

The case (c) is much more extreme. Here at the points to which the transition takes place the upper curve has a far greater slope and the asymptote will be very much below these points. The value of energy of dissociation obtained optically is, therefore, much greater than  $\frac{R}{3}$ .

These observations enable us to review the validity of Saha and Datta's hypothesis.<sup>2</sup> We see that in the case of compound  $\text{SbBr}_3$  where the upper Franck Condon curve is practically horizontal at the point of transition and

therefore the long wavelength limit of absorption gives the true value of the energy of optical dissociation, Saha and Datta's hypothesis<sup>2</sup> is found to be valid. In the case of other compounds the limit of absorption does not give the true value of the energy of optical dissociation, for the upper curve has a much larger slope at the point of transition. The greater the deviation of the limit of absorption from the energy of optical dissociation, the more is Saha and Datta's hypothesis found to be invalid. It might be, therefore, very much possible that Saha and Datta's hypothesis is true and, in the very many reports which we have been having regarding its alleged invalidity, the error might have been made in computing the energy of optical dissociation from the long wavelength limit of absorption by disregarding the effect of the slope of the upper curve at the point of transition.

Coming to the third feature of these results it is found that although the salts examined are bromides and iodides, retransmission is to be found only in the case of  $\text{AsI}_3$  and  $\text{SbBr}_3$ . These appear only at rather high temperatures as found in the case of tinhalides.<sup>4</sup> The difference between the energies of an iodine atom in  $^2P_{3/2}$  and  $^2P_{1/2}$  states is  $7600 \text{ cms.}^{-1}$ , whereas the difference between these limits in the case of  $\text{AsI}_3$  is only  $2402 \text{ cms.}^{-1}$ . It is obvious, therefore, that this difference is not due to the breaking off of the iodine atom in its two metastable states. As we have seen in the case of tin dichloride<sup>5</sup> the retransmissions can also be due to the breaking off of the molecule into residual molecules in different metastable states. The two absorptions are, therefore, probably, due to  $\text{AsI}_3$  breaking up into iodine and  $\text{AsI}_2$  (normal and excited). In the case of  $\text{SbBr}_3$ , the corresponding difference is  $3390 \text{ cms.}^{-1}$ . The difference between the energies of the normal and excited bromine atoms is  $3700 \text{ cms.}^{-1}$ . The two retransmissions may represent the breaking off from the molecule of a normal and an excited bromine atom in the two cases. But the discrepancy between the two differences warns us not to place much reliance on this explanation. The possible explanation should again be sought in the dissociation of the molecule into Br and two dibromides of arsenic (normal and excited). It is curious that in all such polyatomic molecules retransmissions corresponding to the two metastable levels of bromine and iodine are conspicuous by their absence. An explanation of this is, however, still wanting.

#### ACKNOWLEDGMENTS

It is my pleasant duty to acknowledge my indebtedness to Prof. M. N. Saha, for his extreme kindness on the author and for his immense interest in this work. My heartiest thanks are due to Dr. Asundi of Muslim University, Aligarh, for his having taken the microphotograms for me and to Mr Prabhat Kumar Sen-Gupta for his having carried my plates to Aligarh.

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ON NEW TREMATODES OF FROGS AND FISHES OF THE  
UNITED PROVINCES, INDIA.

**Part III.**—On a new Genus *Mehraorchis* and two new Species of *Pleurogenes* (*Pleurogenetinae*) with a systematic Discussion and Revision of the Family *Lecithodendriidae*.

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Communicated by Dr. H. R. Mehra

Received August 2, 1933

***Mehraorchis ranarum*. Nov. gen., nov. spec.**

Host—*Rana cyanophlyctis*

Habitat—The body cavity, usually the pancreatic region.

Locality—Sitapur, Oudh (India)

These interesting distomes are found enclosed in cysts in the body-cavity of—*Rana cyanophlyctis*—a fairly common frog in the ponds of Sitapur district. The cysts, each containing 2–5 parasites, are usually found in the pancreatic region of the host. The maximum frequency of infection is about 40 per cent and the number of parasites varies from 2–14 in a single host. The non-transparent and sluggish-looking worms are of a dirty brown colour with little power of contraction and expansion. The thick ovoid and slightly convex body is beset with straight bluntly-pointed short spines, 0.013 mm. long and 0.005 mm. thick at the base. The spines at the anterior end are thickly crowded but in the post-acetabular region they are smaller in number and far separated from one another. The spines do not project out of the surface of the body. Sexually mature parasites when alive measure 2.4–4.1 mm. in length and 1.3–2.8 mm. in maximum breadth which occurs in the region a little behind the acetabulum. In fixed specimens the dimensions vary from 2.6–4.1 mm. in length and 1.4–2.9 mm. in breadth. The size of the parasites depends largely upon the number enclosed in a cyst. Usually the cysts contain only two parasites which are bigger in size than those in cysts containing a larger number. The suckers are poorly developed and have a spherical outline. The subterminal and ventrally directed oral sucker is 1.2–1.3 times as large as the acetabulum which lies at the junction of the anterior and the middle thirds of the body. A prepharynx is absent. The

muscular pharynx is conical measuring 0.176 mm. in length with an equally broad base. At times it may protrude slightly into the cavity of the oral sucker. A circular constriction divides the lumen of the pharynx into a shorter anterior and a larger posterior chamber. Posteriorly it leads into a fairly long oesophagus, 0.38–0.56 mm. in length and 0.08–0.12 mm. in breadth, which bifurcates at the end of the anterior one-fourth body length. The two wide intestinal caeca extend up to the broad posterior end of the body. Large amoeboid cells line the lumen of the wide intestinal caeca; their nuclei being situated basely and the distal projecting pseudopodia appear largely vacuolated.

The testes are massive and have a roughly triangular or rhomboidal shape usually with an irregular margin. They are situated right and left in the region bounded on each side by the oesophagus, the intestinal caecum and the body-wall. The right testis, 0.56–0.76 × 0.32–0.54 mm. in dimension, is placed a little in advance of the left which is pushed posteriad by the presence of a well-developed cirrus sac situated in front on the same side. The left testis is usually a little smaller than the right and measures from 0.42–0.8 mm. in length and 0.27–0.43 mm. in maximum breadth. The vas efferens of the right testis crosses in front of the ventral sucker to the left side and runs forwards till it unites with the small vas efferens of the left testis to form a rather inconspicuous vas deferens which enters the large and coiled vesicula seminalis. The latter is an inverted U-shaped structure, with a constriction in the middle, occupying the posterior two-fifths of the cirrus sac. The cirrus sac, measuring 0.48–0.6 × 0.12–0.24 mm., is a well-developed, broad, bluntly-pointed, spindle-shaped structure with its extremities slightly curved in opposite directions. It lies obliquely anterior to the left testis extending behind and lying ventrally up to the commencement of the left intestinal caecum. The vesicula seminalis passes anteriorly through a small narrow duct into an elongated conical pars prostatica measuring 0.2–0.3 × 0.05–0.01 mm. Throughout its length the pars prostatica is surrounded by numerous prostate gland cells with prominent rounded nuclei. The anterior curved and narrow part of the cirrus sac encloses a fairly long ductus ejaculatorius which opens into the genital atrium ventrally to the vaginal pore. The small knob-like cirrus is not covered with spines.

A massive ovary of variable shape usually with a markedly crenated outline lies partly overlapping the ventral sucker in the space between the latter and the right intestinal caecum. It measures from 0.26–0.5 mm. in length and 0.26 to 0.45 mm in breadth. A small elongated bulb-shaped receptaculum seminis is situated to the left side of the ventral sucker. The Laurer's canal arises from the neck of the receptaculum seminis just before it opens into the oviduct. The oviduct dilates to form the ootype where it is surrounded by a diffuse mass of shell gland cells. The common vitelline

duct forms a small triangular yolk reservoir which opens into the oviduct immediately before it passes into the ootype. The uterus first runs parallel to the receptaculum seminis and then continues downwards in a more or less straight course in the median line almost parallel to the length of the body. This descending portion of the uterus is filled with sperms and is to be regarded as receptaculum seminis uterinum. The ascending part of the uterus lies in convoluted transverse windings which posteriorly do not extend behind the bifurcation of the excretory bladder; and anteriorly beyond the ovary and the vitellaria. Laterally, however, they extend outside the intestinal cæca reaching almost the body margins. In one specimen in my collection the uterine coils are seen extending as far as the extreme hinder end but this condition is probably due to too much pressure to which the specimen was subjected during fixation. The uterine coils are mostly confined to the ventral half of the body. In its terminal portion the uterus runs straight in an oblique direction from the ventral sucker to join a feebly muscular metraterm which opens into the shallow genital atrium. The vaginal pore is seen lying dorsal to the male genital opening on the left body margin in a slight depression—"the genital atrium." The whole uterus is packed with innumerable eggs of a deep brown colour. The eggs are operculate and measure  $0.03-0.33 \times 0.013-0.015$  mm. The easily eversible shallow genital atrium lies in level with the pharynx.

The vitellaria of the two sides lie laterally confined to the ventral surface of the body underneath the testes and the intestinal cæca, extending slightly inward towards the median line but not meeting each other. The right vitelline gland lies a little anterior to that of the left side, extending from the middle of the oesophagus to a little distance behind the posterior margin of the acetabulum. Inwardly also it spreads further towards the median line partly covering the ovary. The left vitelline gland occupies the region from behind the obliquely placed cirrus sac to a short distance behind the ventral sucker. The vitellaria never extend backwards to overlap the uterine coils. Each gland consists of a number of closely scattered bunches of grapelike follicles. Small vitelline ducts arise from these groups and fuse to form one large duct on each side. The vitelline duct of each side runs towards the median line and unites with its fellow of the other side near the ventral sucker to form a yolk reservoir which, as mentioned before, opens into the oviduct through a small common vitelline duct.

The excretory bladder is somewhat Y-shaped with the stem of a smaller length than the cornua; its shape approaches somewhat midway between that of latters Y and V. The main stem bifurcates anteriorly at  $0.32-0.48$  mm. from the posterior end. It measures  $0.27-0.43$  mm. in length and has a funnel-shaped outline with the greatest breadth of  $0.16$  mm. at its anterior margin. At its anterior corners arise the cornua, one on each side, extending



as far forward as the ventral sucker. The terminal part of the main stem is surrounded by deeply staining parenchymatous cells which form a sphincter around the opening. The excretory opening lies subterminally at the posterior end on the ventral side of the body.

This interesting parasite is assigned to the subfamily *Pleurogenetinae* Looss as defined by Mehra and Negi 1928, with which it presents unmistakable affinities on account of the topography of the genital organs and the position of the genital pore on the left body margin. The excretory bladder, though somewhat different, can, nevertheless, be derived from the typical V-shaped condition characteristic of the subfamily, by the union of the arms for some length forwards to form a moderately long main stem. Amongst the known genera of the *Pleurogenetinae* it resembles *Pleurogenes* in the form of the body, the relatively great length of the intestinal cæca and its occurrence in cysts (*P. arcanus* Klein 1905 occurs encysted in the liver, pyloric region and neck of urinary bladder of frogs). It resembles somewhat the genus *Prosotocus* in the position of its testes which lie one on each side in the anterior part of the body, but the left testis, however, lies behind the cirrus sac and not in front of it as in *Prosotocus*. The vitellaria are confined, as in the genus *Ganeo*, to the ventral half of the body. In all other important features this species does not fit in with any of the known genera, therefore, a new genus is created for its reception. The genus is named after Dr. H. R. Mehra to whom my respectful gratitude is due, for his invaluable help and advice.

*Generic Diagnosis*—*Pleurogenetinae*, small, fleshy, ovoid with flattened elliptical cross section; integument spiny, spines sparsely distributed in post-equatorial region. Suckers small, slightly muscular, acetabulum smaller than the oral sucker lying at the junction of anterior and middle thirds of body-length. Prepharynx absent; pharynx well-developed and muscular; oesophagus moderately long; intestinal cæca extending nearly up to the posterior end. Testes massive; asymmetrically situated in the anterior body to the right and left in the space between oesophagus, intestinal cæca and body-wall. Ovary to the right side, partly overlapping the ventral sucker. Vitellaria, confined to the ventral half in the anteriolateral region extending almost to the median line, composed of a large number of grape-like bunches. Laurer's canal and receptaculum seminis present; shell gland diffuse, situated close to the acetabulum. Genital atrium shallow, easily eversible; situated on a level with the pharynx on the left body margin; male and female openings separate. Excretory bladder with a somewhat prominent stem and longer cornua; excretory pore subterminal and ventral. Muscular cirrus sac situated obliquely, anterior to left testis; containing well-developed coiled vesicula seminalis, pars prostatica and ductus ejaculatorius. Uterus post-ovarian, not extending behind blind ends of intestinal cæca; metraterm present. Ova

numerous small, operculate, measuring  $0.03-0.033 \times 0.013-0.015$  mm. in size.

Encysted in the body cavity, especially in the pancreatic region of Amphibia.

### Genus *Pleurogenes*, Looss, 1896

This is the oldest genus belonging to the subfamily *Pleurogenetinae* Looss 1899, the type species of which was described by Rudolphi in 1819 as *Distoma clavigerum*, parasitic in the intestine of Anurans. V. Linstow in 1888 described the same parasite under the name *Dist. neglectum*. Pagenstecher in 1857 and Pachinger in 1888 described with figures a distome similar to that described by Rudolphi and gave it the same name. Olsson described the specimens of *Dist. medians*, which he found in the intestine of Anurans in 1876. Sonsino also described the same form in 1894 as *Dist. tacapense*, which Looss in 1898 renamed as *Dist. tacapensis*. Looss in his well-known paper published in 1899 pointed out the identity of *Dist. clavigerum* Rud. with *Dist. neglectum*, V. Linstow and of *Dist. medians* Ols. with *Dist. tacapense* Sons and *Dist. tacapensis* Lss. The only representative of the genus *Pleurogenes*, parasitic in the Reptilia was described in 1896 independently by Looss and Sonsino, as *Dist. tacapense* from the intestine of *Chamaeleo basiliscus*. Two years later Looss gave this species its present name of *Pleurogenes tener* and in the following year transferred it to the genus *Prosotocus*. Stafford in 1905 following Looss retained it under *Prosotocus*, but Klein, in the same year, however, transferred it back to the genus *Pleurogenes*, adding two more new species to the genus, i.e. *P. sphericus* from the intestine of an Indian frog *R. hexadactyla* and *P. arcanus* found encysted in the liver, pyloric region and around the neck of the urinary bladder of some members of *Ranidae*. The latter species was first described by Stafford in 1900 as *Dist. medians* Ols. and almost at the same time by Nickerson as *Dist. arcanum*. Four years later Stafford created a new genus *Loxogenes* for *Dist. arcanum*. Mehra and Negi in 1928, following Klein, have referred it to the genus *Pleurogenes* in their key. Tubangui in 1928, Fuhrmann in 1928, Travassos in 1930 and Krull in 1933 have, however, retained the genus *Loxogenes*. As will be seen from the discussion the genus *Loxogenes* cannot be maintained. *P. gastroporus*, parasitic in the intestine of *R. cyanophlyctis* India, was described by Luhe in 1901 and a new variety of it *var. equalis* from the intestine of *R. tigrina* was added by Mehra and Negi in 1928. Johnston in 1912 described *P. freycineti* and *P. solus* from the intestine of Australian tree frogs, *Hyla freycineti* and *H. aurea*. Travassos in 1921, following the suggestion made by Stafford in 1904 split up the genus *Pleurogenes* Lss. into two groups on the basis of the length of the intestinal cæca. Group one, comprising of the species with intestinal cæca extending beyond the ventral

sucker he retained as the genus *Pleurogenes*; whereas for the species in which the cæca are short, never extending beyond the centrum of the acetabulum, he created a new genus *Pleurogenoides*. Mehra and Negi in 1928 divided the genus *Pleurogenes* Lss. into two sub-genera—*Pleurogenes* and *Telogonella*—on the basis of the length of the intestinal cæca and the position of the genital pore. In the sub-genus *Pleurogenes* the intestinal cæca do not extend posteriorly beyond the centrum of the acetabulum and the genital pore is more cephalad, while in *Telogonella* the cæca extend posteriorly beyond the acetabulum and the genital pore is not so anteriorly situated. In his papers published in 1930 and 1931 Travassos reaffirms the validity of his genera *Pleurogenes* and *Pleurogenoides*. The study of intermediate species between the two genera created by Travassos, such as *P. orientalis* n. sp., *P. lobatus* Ozaki 1926 and *P. intermedius* Isaitschikow 1926 (Travassos did not consult this paper) has convinced me of the untenability of the genus *Pleurogenoides* Trav., which I accordingly drop, referring all its species back to *Pleurogenes* Looss. Ozaki in 1926 described *P. lobatus* from the bile ducts of Japanese frogs—*Polypedates buergeri*. Two years later Tubangui found a species *P. taylori* in the intestine of *R. vittigera* in Philippine. In 1930 Travassos described *P. stromi* from the intestine of *R. esculanta* and Africa *P. loossi* from the intestine of the same host. The latest addition to the list of species of this genus is *P. minus* parasitic in the intestine of Pike—*Esox lucius*, described by Pigulewsky in 1931. I add in this paper two new species to this genus from the gut of *R. cyanophlyctis* (Northern India).

***Pleurogenes orientalis*.—n. sp.**

Host—*Rana cyanophlyctis*.

Habitat—Intestine.

Locality—Sitapur, Oudh (India).

In its frequency of occurrence this species is as rare as *Prosotocus infrequentum* Srivastava 1933. In August 1932 only two frogs were found infected with one parasite each, out of over a hundred specimens of *R. cyanophlyctis* examined. The distomes show little power of contraction and expansion and are of a light brown colour. The thin and transparent body is ovoid in form with broadly rounded ends and is studded with minute backwardly directed spines except in the region behind the excretory opening. In balsam mounts the parasites measure 1.4–1.6 mm. in length and 0.96–1.1 mm. in maximum breadth across the acetabular region. The suckers are fairly large and muscular with spherical outline. The oral sucker, 0.27–0.28 mm. in diameter, is larger than the acetabulum and is situated on the ventral surface a little behind the anterior end. The acetabulum lies for the greater part of

its diameter in the posterior half of the body measuring 0.22 mm. in diameter. The size ratio of the oral and the ventral suckers is 4:3.

The genital opening is situated on the left body margin in level with the junction of the anterior and middle thirds of the oral sucker. The excretory pore is subterminal, situated on the ventral surface a little in front of the hinder end. A muscular pharynx,  $0.064 \times 0.012$  mm. in size, lies at the base of the oral sucker. The oesophagus being entirely absent the pharynx is immediately followed by the intestinal bifurcation which lies 0.3 mm. in front of the acetabulum. The intestinal caeca at first run more or less horizontally, one on each side and then continue their downward course, laterally near the body-wall, terminating a little behind the acetabulum, just in front of the posterior third of body length.

The testes lie symmetrically near the lateral body-walls, one on each side, immediately behind the blind extremities of the intestinal caeca about the junction of the middle and posterior thirds of the body length. The right testis is nearly spherical in shape, measuring 0.19 mm. in diameter, while the left testis,  $0.14 \times 0.17$  mm. in size, is more or less ovoid in outline. The vasa efferentia, which arise as delicate tubes from the anterior margin of each testis, run forwards separately for a short distance before they unite in the median line close in front of the acetabulum to form a small vas deferens which soon enters the cirrus sac. The cirrus sac is well developed and situated to the left side, ventrally to the left intestinal caecum, extending from the anterior margin of the acetabulum to the level of about anterior one-third diameter of the oral sucker. It is quite large for the size of the distome, measuring 0.72 mm. in length, and consists of a basal horizontal club-shaped part and a narrow tubular vertical part. The angle of curvature between these two parts is a sharp right angle. The basal club-shaped part encloses a coiled vesicula seminalis of 0.32 mm. length and a fairly long pars prostatica which becomes narrower towards its terminal end, where it passes into the ductus ejaculatorius of 0.3 mm. length. The cirrus sac opens terminally into the inconspicuous genital atrium which lies on the left body margin.

The ovary, 0.13 mm. in diameter, is spherical and is situated to the right side about the end of the first body-half, close inside and in contact with the right caecum, at about the level of the anterior half of the acetabulum. The oviduct arises from the middle of its inner margin and is joined after a short length by a flask-shaped receptaculum seminis of  $0.13 - 0.15 \times 0.08 - 0.1$  mm. size which lies obliquely to the right side of the acetabulum between it and the ovary. As in the other species of *Pleurogenetinae* a small Laurer's canal arises from the receptaculum seminis. The yolk reservoir, the shell gland mass and the receptaculum seminis are all lodged in the space between the ovary and the acetabulum.

The vitellaria are well developed and confined to the anterior half of the body. The follicles are scattered mesially between the oral sucker and the acetabulum, running into one another at a few places, but they are much aggregated laterally extending from the middle of the oral sucker to the level of about the middle of the acetabulum.

The uterus arises from the right side of the acetabulum, passes downwards and forms in the post-acetabular region a compact convoluted mass filling a little more than the posterior one-third of the body. The ascending part of the uterus passes distally to the left side of the acetabulum, running parallel and outer to the cirrus sac, before it terminates into a feebly muscular metraterm. The metraterm opens in the inconspicuous genital atrium to the left side of the male opening. The uterus is packed with numerous small operculate eggs of a light brown colour measuring  $0.023 \times 0.013$  mm. in size.

The excretory bladder is V-shaped with its cornua extending anteriorly up to the posterior margin of the testes. The excretory pore lies on the ventral surface, 0.13 mm. distance in front of the posterior end of the body.

This interesting species differs remarkably in many features from all the other species of the genus *Pleurogenes* Lss. It resembles somewhat *P. gastroporus* and *P. gastroporus* var. *equalis* in the shape of its body, shape of the excretory bladder, position of the excretory pore and the uterine convolutions being confined to the post-acetabular region. But it differs from them in such important features as the relative position and size ratio of the suckers, absence of the œsophagus, relative length of the intestinal cæca, shape and disposition of the vitellaria, topography of the gonads, the shape and position of the cirrus sac and the position of the genital pore.

#### ***Pleurogenes sitapurii*.—n. sp.**

Host—*Rana cyanophlyctis*.

Habitat—Duodenum.

Locality—Sitapur, Oudh (India).

The parasites belonging to this species are the smallest of all the distomes infecting *Rana cyanophlyctis*. They were first met with about the middle of July 1932. During the rainy season (July—September) the frequency of infection is about 40 per cent varying in intensity from 6—25, but with the approach of winter it gradually declines. The parasites are usually found attached to the wall of the descending part of the duodenum. Only once they were found throughout the length of the duodenum. In their natural habitat they appear as minute dust particles, rendered conspicuous by the colour of the contained ova. They are so delicate that they can hardly bear the weight of even a small glass coverslip. They appear to be extremely susceptible to changes in diet and temperature, as they cannot live for more than an hour in any

nutritive solution. In the living condition they are grey in colour and show little power of contraction and expansion, measuring 0.78–0.94 mm in length and 0.46–0.64 mm. in maximum breadth which lies across the acetabulum. The body is comparatively thick and presents an oval outline, narrower anteriorly and broader and somewhat rounded off behind. In entire mounts the size varies from 0.6–0.96 mm. in length and 0.35–0.5 mm. in maximum breadth, according to the state of contraction. The cuticle is covered all over with fairly large and pointed spines of 0.013 mm. length.

The suckers are feebly muscular and have a circular outline. The oral sucker is subterminally situated on the ventral surface, measuring 0.11–0.13 mm. in diameter. The acetabulum, 0.11–0.14 mm. in diameter, *i.e.*, nearly equal to or slightly larger than the oral sucker, is situated in the posterior half of the body, just in front of the hinder third of body length.

The genital atrium, containing the male and the female openings, is situated on the left body margin in level with the pharynx. The excretory opening is situated on the ventral surface a little in front of the posterior extremity.

The muscular globular pharynx of 0.03–0.065 × 0.05–0.07 mm. size is followed by a short oesophagus of 0.06–0.1 mm. length which bifurcates just in front of the ovary, in level with the junction of the pars prostatica and the ductus ejaculatorius. The intestinal caeca, usually of equal length, are short with moderately divergent extremities, ending blindly much in front of the acetabulum. They rarely extend beyond the posterior ends of the ovary and the cirrus sac.

The testes are massive structures, rounded or ovoid in shape, situated somewhat asymmetrically one on each side immediately in front of the centrum of the acetabulum. The right testis, 0.1–0.23 × 0.08–0.18 mm. in size, is usually a little larger than the left testis and is separated from the right body margin by the anteriorly passing coils of the uterus. The left testis, 0.1–0.23 × 0.08–0.1 mm. in size, is pushed somewhat caudad by the well-developed cirrus sac which lies in front and is separated from the latter and the acetabulum by the outgoing coils of the uterus. The vasa efferentia arise as delicate tubes, one from the posterior inner end of each testis, and run more or less transversely in front of the acetabulum to enter the vesicula seminalis through a small and inconspicuous vas deferens. The cirrus sac, 0.3–0.36 × 0.06–0.09 mm. in size, is highly muscular and has a slight S-shaped curvature. The coiled vesicula seminalis is divided by a prominent constriction into two distinct parts, *i.e.*, a basal swollen sac-shaped part of 0.11 × 0.08 mm. size and an anterior tubular part of 0.08 mm. length and 0.02 mm. breadth. The pars prostatica is elongated and somewhat flask-shaped, measuring 0.1–0.16 × 0.03–0.04 mm. in size. It narrows anteriorly to form the ductus ejaculatorius of 0.06–0.1 mm. length which is followed by a knob-like cirrus of 0.013 mm. length.

The ovary,  $0.09-0.18 \times 0.05-0.13$  mm. in size, is situated somewhat in the median line, more to the right side, far in front of the acetabulum and close behind the intestinal fork partly overlapping the right intestinal caecum. Usually it has a regular outline with entire margin but sometimes it is lobed. The receptaculum seminis,  $0.1 \times 0.04$  mm. in size, is flask-shaped, situated transversely or obliquely, to the right side, close in front of the acetabulum. The Laurer's canal,  $0.02$  mm. in diameter, arises as a curved tube from the neck of the receptaculum seminis and runs posteriorly to open on the mid-dorsal surface in the region of the acetabulum.

The vitellaria are composed of usually an equal number of 4-8 large, pear-shaped follicles, each of  $0.05-0.09 \times 0.02-0.05$  mm. size. The left vitelline gland lies close to the median line, behind the intestinal bifurcation, in the space between the cirrus sac and the ovary. The right vitelline gland lies close to the right body margin and slightly cephalad to the left gland. The two vitelline ducts run posteriorly and unite together to form the common vitelline duct to the right side of the ventral sucker. The common vitelline duct travels forward to form the yolk reservoir situated in front near the junction of the receptaculum seminis with the oviduct, in the space between the ovary and the acetabulum. A diffuse shell gland mass lies median, just in front of the acetabulum.

The descending part of the uterus commences near the left side of the acetabulum and forms several longitudinal and transverse loops, extending from the left testis to the posterior end of the body. Behind the acetabulum it passes into a transverse loop which extends forwards to the right side in the form of a longitudinal loop right up to the pharynx and then turns downwards forming two or three loops near the hinder end on the same side before it crosses over again to the left side to join the metraterm of  $0.15 \times 0.018$  mm. size, which crosses the cirrus sac to lie close to its right side. In fully mature specimens, the coils of the uterus as described above are indistinguishable and the whole uterus appears as a mass of eggs. The eggs are dark brown in colour, oval and operculate, measuring  $0.023-0.025 \times 0.013$  mm. in size.

The V-shaped excretory bladder consists of two broad cornua and a very small median stem. Both the cornua extend up to the level of the acetabulum and are slightly constricted in the middle of their length. The excretory pore lies on the ventral surface a little in front of the posterior end.

Of all the species of the genus *Pleurogenes* Looss *P. sitapurii* n. sp. is closely allied to *P. solus* Johnston 1912 in the shape and size of its body, position of the suckers and the genital pore and in the vitelline glands being composed of a few large follicles. It differs, however, from *P. solus* in the length of the oesophagus and the intestinal caeca, position and size of the gonads, size and number of the vitelline follicles, characteristic arrangement of the uterine coils and the position of the excretory pore.

### Systematic Discussion on the Genus *Pleurogenes*, Looss, 1896.

The genus *Pleurogenes* Lss. is parasitic in amphibia except *P. tener* which is described from a reptile—*Chamaeleo basiliscus* and *P. minus* which is parasitic in the intestine of a fish—*Esox lucius*. While the position of *Pleurogenes* as the typical genus of the sub-family *Pleurogenetinae* needs no discussion a revision of its scope seems to be necessary.

Stafford in 1904 established the genus *Loxogenes* for *Dist. arcanum* Nickerson 1900 "on account of its genital opening being situated on the ventral surface, midway between the left intestinal caecum and the body margin." This is the only feature which separates *Loxogenes* from *Pleurogenes*. But the genital pore does not lie exactly on the body margin in several other species of *Pleurogenes* such as *P. sphaericus* and *P. intermedius*, in which it occupies a position far inwards to the left body margin. It is, therefore, necessary to drop this genus which contains species closely related to all the other species of the genus *Pleurogenes*.

Travassos in 1921, according to the suggestion made by Stafford in 1904, created a new genus *Pleurogenoides* for such species of the genus *Pleurogenes* as have short intestinal caeca (never extending behind the acetabulum) and retained the genus *Pleurogenes* for *P. claviger*. In his papers published in 1930 and 1931 he has maintained *Pleurogenes* and *Pleurogenoides* as distinct genera. Two genera can be accepted only so long as the generic differences between them are of absolute value and the intermediate forms connecting them do not exist. As the genera *Pleurogenes* and *Pleurogenoides* are now connected by such intermediate species as *P. orientalis* n. sp. *P. intermedius* Isaitschikow and *P. lobatus* Ozaki it appears necessary to drop the genus *Pleurogenoides*. I accordingly drop the genus *Pleurogenoides* and assign the species belonging to it back to the genus *Pleurogenes* Looss.

In splitting up the genus *Pleurogenes* into two subgenera Mehra and Negi have recognized the differences between the two groups of species, without losing sight of their close relationships. It is certainly a convenient arrangement which provides for a systematic grouping of a large number of species belonging to one genus. It may be pointed out that *P. orientalis* n. sp. is one of the intermediate species which connects the two subgenera *P. 'Pleurogenes'* and *P. (Telogonella)*.

In the light of our recent knowledge of the genus the diagnosis of *Pleurogenes* as given by Mehra and Negi needs a certain amount of modification.

The amended diagnosis is as follows:

*Diagnosis*.—Body oval, elliptical, oblong or somewhat spherical; size small; acetabulum usually situated about the middle of body rarely in front or behind it. Oesophagus absent, short or long; length of intestinal caeca



extremely variable. Genital pore situated on ventral or dorsal surface, near or on the left (rarely dextral) body margin, in front of or in level with intestinal bifurcation except in *P. lobatus* where it is behind it. Testes two, regular, except in *P. lobatus* (lobed), situated symmetrically, one on each side, or obliquely one behind the other, in level with, in front of or behind the acetabulum. Ovary regular or lobed, usually pretesticular rarely in level with the testes, dextral or median, near or in front of the acetabulum. Cirrus sac enclosing coiled vesicula seminalis and well-developed pars prostatica present. Vitelline follicles confined to the anterior body half. Uterine convolutions usually confined to the posterior half, rarely extending up to the level of the pharynx. Excretory bladder V-shaped with or without a short median stem or Y-shaped with the main stem much longer than the cornua as in *P. bicolor* Krull 1933; excretory opening terminal or subterminal. Eggs small, and numerous, measuring,  $0.02-0.037 \times 0.01-0.016$  mm. in size.

Host—usually amphibia only exceptionally fishes and reptile

Habitat—the gut of the host except *P. arcannum* which lives in cysts around the pylorus, surface of liver and neck of the urinary bladder of frogs.

### Key to the Sub-genera of *Pleurogenes*, Looss 1896.

- Intestinal cæca confined to the first half of body  
and not extending behind the acetabulum ... Sub-genus *Pleurogenes*.
- Intestinal cæca extending behind the acetabulum  
into the posterior half of body ... Sub-genus *Telogonella*.

### Key to the Species of the Sub-genus *Telogonella*

1. The main stem of the excretory bladder much longer than the cornua ... *P. bicolor*.  
The main stem of the excretory bladder much smaller than the cornua ... 2
2. Gonads lobed ... *P. lobatus*.  
Gonads with entire margins ... 3
3. Testes obliquely situated, one behind the other ... *P. loossi*.  
Testes symmetrically situated, one on each side ... 4
4. Testes overlapping the cæca and situated a little in front of their blind ends ... *P. intermedius*.

- Testes situated behind the termination of the intestinal cæca ... .. 5
5. Intestinal cæca end a little behind the acetabulum, in front of the posterior third body-length ... .. *P. orientalis*.
- Intestinal cæca extend far behind the acetabulum, never ending in front of the  $\frac{2}{3}$ th bodylength ... .. *P. claviger*.

### Key to the Species of the Sub-genus *Pleurogenes*

- Acetabulum distinctly post-equatorial ... A
- Acetabulum equatorial or pre-equatorial .. B
- A. Oesophagus absent ... .. *P. sphericus*.
- Oesophagus present ... .. 1
1. Intestinal cæca extend up to the acetabulum *P. solus*.
- Intestinal cæca do not extend up to the acetabulum ... .. 2
2. Excretory pore terminal ... .. *P. tener*.
- Excretory pore subterminal ... .. *P. sitapurii*.
- B. Ovary extra-cæcal ... .. 1
- Ovary not extra-cæcal ... .. 2
1. Intestinal cæca extend up to the acetabulum *P. minus*.
- Intestinal cæca stop in front of the acetabulum *P. medians*.
2. Oesophagus present ... .. 3
- Oesophagus absent ... .. 4
3. Genital atrium opens on the left body margin *P. freycineti*.
- Genital atrium opens subterminally half way between the left intestinal cæcum and body margin ... .. *P. arcanum*.
4. Testes situated anterior to the ends of the intestinal cæca ... .. *P. taylori*.
- Testes lie behind the ends of the intestinal cæca 5
5. Vitellaria consist of a few large follicles; pre-cæcal ... .. *P. stromi*.
- Vitellaria consisting of a large number of small follicles scattered all over the cæca and meeting in the median line ... 6
6. Acetabulum larger than the oral sucker ... *P. gastroporus*.
- Acetabulum of the same size as the oral sucker ... .. *P. gastroporus* var. *equalis*.

### Systematic Discussion and Revision of the Family *Lecithodendriidae*. Odnher 1911.

Looss in 1896 created the subfamily *Lecithodendriinae* to include the genera *Lecithodendrium*, *Phanaropsolus* and *Pycnoporos*. Later in 1899 he considered the resemblance between *Lecithodendrium* and *Brachycaelium* to be so close that he dropped the subfamily *Lecithodendriinae* and assigned all the genera to the subfamily *Brachycaeliinae* Looss. Luhe in 1909 adopts the latter course in the "Susswasserfauna Deutschlands". Two years later Odnher reshuffled the whole arrangement and removed all the genera except *Brachycaelium* from the *Brachycaeliinae* to the *Lecithodendriinae* which then is the *Brachycaeliinae* minus *Brachycaelium*. The *Brachycaeliinae*, containing *Brachycaelium*, was assigned by him to the family *Dicrocoelidae* Cort 1919, Mehra and Negi 1926 and Mehra 1931 found this position untenable and assigned this subfamily to the family *Lepodermatidae*.

The subfamily *Pleurogenetinae* was established by Looss in 1899 to include the genera *Pleurogenes* and *Prosotocus*. The *Pleurogenetinae* shows unmistakable affinities with the *Lecithodendriinae* in the presence of a V-shaped excretory bladder, position of the acetabulum about the middle of the body, situation of the ovary near it to the right side or about the median plane and the disposition of the uterine convolutions. Further, the absence of a cirrus sac in *Ganeo* brings the *Pleurogenetinae* closer to such genera of the *Lecithodendriinae* as *Lecithodendrium*, *Prosthodendrium*, *Acanthatrium* and *Pycnoporos*, etc. The important differences between the two subfamilies lie in the position of the genital pore and the host. Odnher in 1911 recognised the close affinities between the two subfamilies and brought them together under one and the same family *Lecithodendriidae*. Though this view of the relationship has never been questioned, the recent work of Fuhrmann in 1928 and of Travassos in 1922, 1928, 1930 and 1931 has caused some confusion in the limits of the two subfamilies owing to the unnatural grouping of the genera under them. Unfortunately both these authors have not given any indication of the reasons for assigning the various genera to the two subfamilies.

Fuhrmann 1928 includes under *Lecithodendriinae* the genera *Lecithodendrium*, *Pycnoporos*, *Phanaropsolus*, *Parabascus*, *Mesodendrium*, *Acanthatrium*, *Limatulum* and *Castroia*, and under the *Pleurogenetinae* the genera *Pleurogenes*, *Loxogenes*, *Prosotocus*, *Mosesia*, *Postorchigenes*, *Brandesia*, *Pleurogenoides*, *Ganeo*, *Eumogacetes* and *Anchitrema*. Travassos in 1922 while describing a new species of *Eumagacetes*—*E. perodiosus* from the cloaca of *Pioya cajana*, Brazil—raised the genus to the rank of a family—*Eumagacetidae*. In 1928 he included the genera *Lecithodendrium*, *Paralecithodendrium*, *Acanthatrium* and *Castroia* under *Lecithodendriinae*. In 1930 under the *Pleurogenetinae* he included the genera *Pleurogenes*, *Pleurogenoides*, *Cryptotrema*, *Limatulum*, *Loxogenes*, *Prosotocus*

*Brandesia*, *Phanaropsolus*, *Moesia* and *Parabascus*. In the last paper he also referred the genus *Ganeo* to the subfamily *Lecithodendriinae*.

From the above lists it appears that the classification of the family *Lecithodendriidae* is entirely arbitrary and that a revision of the family is needed.

The genera *Mesodendrium* Faust 1919 and *Lecithodendrium* Looss 1896 have already been referred as synonymous by Dollfus in 1931. The untenability of the genera *Loxogenes* Stafford and *Pleurogenoides* Travassos has been made clear in the systematic discussion before and the genus *Ganeo* has been already assigned by me 1933 to the *Pleurogenetinae*. The family *Eumagacetidae* Travassos 1922 based on the length of the intestinal cæca and the extent of the vitellaria—characters which vary within wide limits in the family *Lecithodendriidae*—cannot be maintained. I, therefore, refer the genus *Eumagacetes* to the *Lecithodendriinae* in which it was first included.

The classification of the genera of the *Lecithodendriidae* into subfamilies should not be based on (a) the length of the intestinal cæca, (b) the extent of the vitellaria, and (c) the position of the testes because they are very variable in this family. The only features which show constancy and have little variability are the position of the genital pore and the host. The length of the intestinal cæca varies widely in such closely related genera as *Eumagacetes*, *Lecithodendrium*, *Prosotocus*, *Mehraorchis* and *Pleurogenes* and even in the different species of the last genus; so does also the extent of vitellaria in *Lecithodendrium*, *Prosotocus*, *Prosthodendrium*, *Eumagacetes*, *Pleurogenes*, *Mehraorchis* and *Cryptotropa*. The position of the testes also presents the same condition in different genera. But the position of the genital pore, sinistral, dextral or median and the host are the features which should be recognised as the basis of the division into the two subfamilies. Those genera which have the genital pore situated to the left (exceptionally to the right) of the median line either in front or in the neighbourhood of the acetabulum and are usually parasitic in fishes, amphibia and reptiles are separated under the subfamily *Pleurogenetinae* and the others which have the genital pore situated in or about the median line and are parasitic in reptiles, birds and mammals are included under the *Lecithodendriinae*. The genus *Parabascus* which has the genital pore to the left side and is parasitic in bats combines the characters of both the subfamilies. In this case I should point out that the nature of the host should not be considered of very great importance, as it is probable that the insect larvæ through which the larval stages of the members of the *Lecithodendriidae* are passed, form the food common to hosts of different groups such as amphibia and bats. For similar reasons Braun also cautioned against attaching undue importance to widely different hosts of closely related species, i.e., *Crepidostomum laureatum* Zedar, parasitic in fish and *C. mitocetus*, parasitic in bats.

In view of the several new forms which have been described in recent years the diagnosis of the family *Lecithodendriidae*, as given by Odhner in 1911 needs to be modified and the scope of its subfamilies redetermined.

**Emended Diagnosis of Lecithodendriidae, Odhner, 1911**

Body small rounded or fairly long and elongated; ventral sucker in the middle of the body or not far from it; spines present or absent. Prepharynx present or absent; pharynx present; oesophagus present or absent; intestinal caeca of varying length. Testes situated usually symmetrically, one on each side, rarely obliquely one behind the other in different regions of the body. Cirrus sac present or absent or represented by a pseudocirrus sac. Ovary usually situated to the right side, sometimes in the median line, not far from the ventral sucker; a small receptaculum seminis and Laurer's canal present. Vitellaria of variable extent, consisting of only a few or numerous follicles, confined either to definite regions or scattered all over. Uterus strongly convoluted. convolutions usually confined to the postacetabular region, rarely extending as far forwards as the pharynx; eggs numerous, measuring 0.015–0.06 mm in size. Excretory bladder V-shaped with or without a short median stem, rarely Y-shaped with the main stem larger than the cornua.

Parasitic in insect eating vertebrates, from fishes to mammals.

**Subfamily—Lecithodendriinae, Looss char. emend.**

Body elongated or rounded, moderately large or small, suckers well developed; spines present or absent. Intestinal caeca only exceptionally extend beyond the acetabulum as in *Eumagacetes* and *Anchitrema*. Testes more or less symmetrically situated; genital pore median, situated in front or in the neighbourhood of the acetabulum. Eggs small and numerous, 0.017–0.03 mm. in size. Excretory bladder V-shaped.

Parasitic in reptiles, birds and mammals.

**Subfamily—Pleurogenetinae, Looss, char, emend.**

Body elliptical, elongated, oval or rounded, size small; suckers not particularly muscular; body partly or wholly beset with spines. Intestinal caeca of variable length. Testes variable in position, usually symmetrically situated, one on each side; rarely obliquely placed one behind the other; cirrus sac or pseudocirrus sac present or absent. Genital pore sinistral, rarely dextral, marginal or submarginal; in front or in the neighbourhood of the acetabulum. Vitellaria of varying extent. Uterus much convoluted, generally confined to the postacetabular region, exceptionally extending as far forwards as the pharynx; metraterm present or absent. Excretory bladder V-shaped with or without a short median stem, exceptionally Y-shaped. Eggs usually of deep brown colour, 0.02–0.06 mm. in size.

Parasitic in fishes, amphibia and reptilia except *Parabaszus* which is found in bats.

Family—*Lecithodendriidae* Odhner 1911.

Subfamily—*Lecithodendriinae* Looss 1896.

Genera—1. *Lecithodendrium* Looss 1896.

2. *Pycnoporus* Looss 1899.

3. *Phanaropsolus* Looss 1899.

4. *Anchitrema* Looss 1899.

5. *Eumagacetes* Looss 1899.

6. *Acanthatrium* Faust 1918.

7. *Limatulum* Travassos 1926.

8. *Castroia* Travassos 1928.

9. *Mosesia* Travassos 1928.

10. *Prosthodendrium* Dollfus 1931.

Subfamily—*Pleurogenetinae* Looss 1899.

Genera—1. *Pleurogenes* Looss 1896.

2. *Prosotocus* Looss 1899.

3. *Brandesia* Stossich 1899.

4. *Ganeo* Klein 1905.

5. *Parabascus* Looss 1907.

6. *Cryptotropa* Strand 1931.

7. *Postorchigenes* Tubangui 1928.

8. *Mehraorchis* Srivastava 1933.

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#### EXPLANATION OF PLATES

Fig. 1—Ventral view of *Mehraorchis ranarum*.

Fig. 2—Do. Do. *Pleurogenes orientalis*.

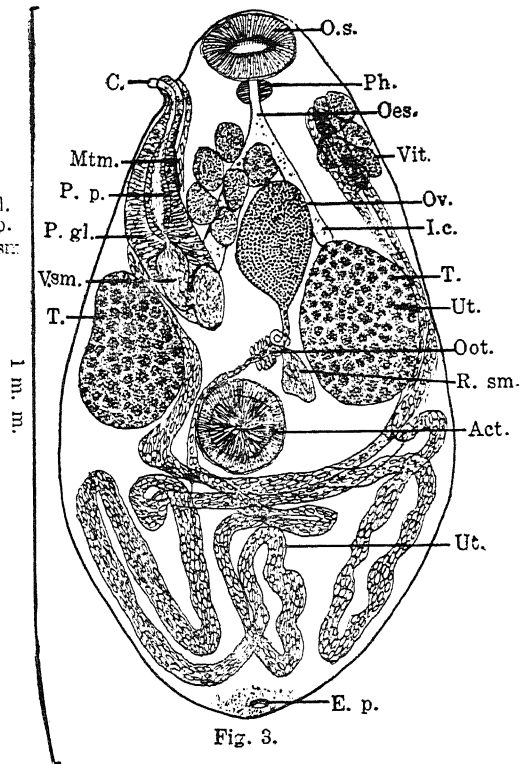
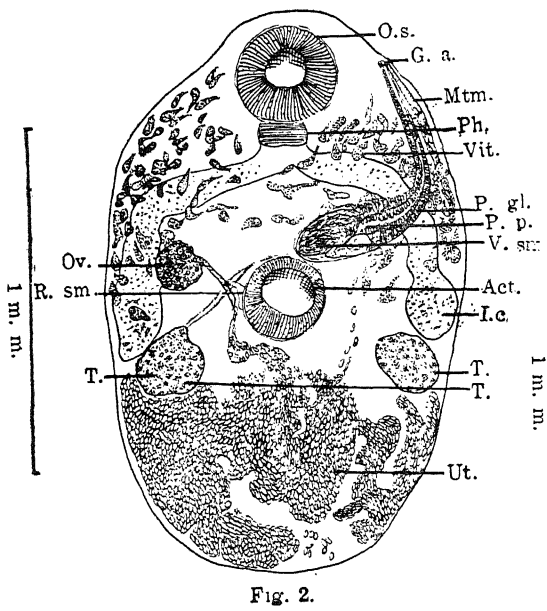
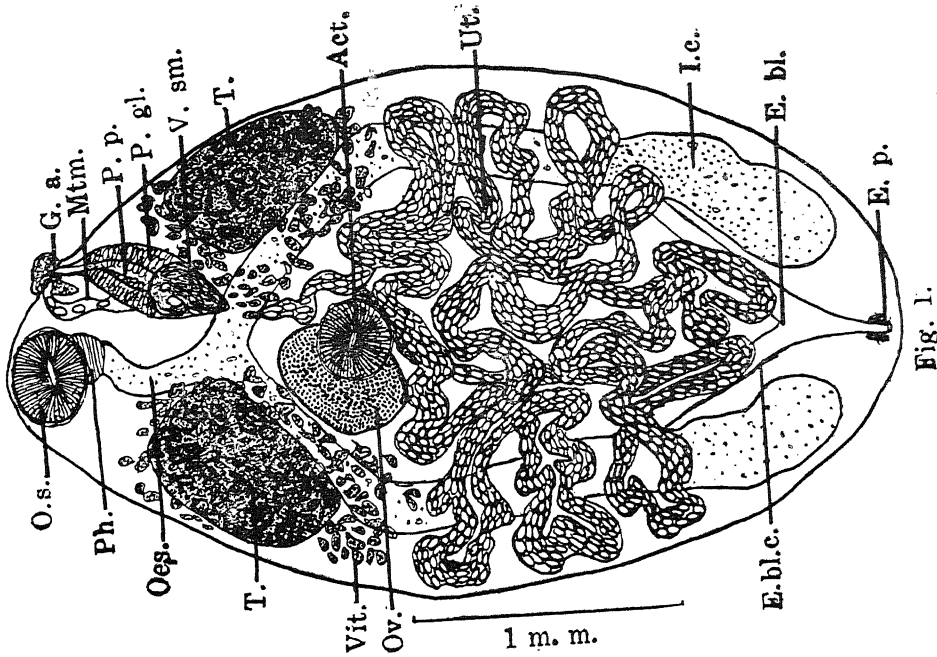
Fig. 3—Dorsal view of *Pleurogenes sitapuri*.

#### LETTERING

Act	...	Acetabulum.	Oes.	...	Oesophagus.
C	...	Cirrus.	Oot.	...	Ootype.
E. bl.	...	Excretory bladder.	O. s.	...	Oral sucker.
E. bl. c.	...	Cornua of Excretory bladder.	Ph.	...	Pharynx.
E. p.	...	Excretory pore.	P. gl.	...	Prostate glands.
G. a.	...	Genital atrium.	R. sm.	...	Receptaculum seminis.
I. c.	...	Intestinal caecum	T.	...	Testis.
Mtm.	...	Metraterm.	Ut.	...	Uterus.
Ov.	...	Ovary.	V. sm.	...	Vesicula seminalis.
P. p.	...	Pars prostatica.	Vit. d.	...	Vitelline duct.

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# ON THE $\beta$ -RAY ACTIVITY OF RADIOACTIVE BODIES

(Preliminary Communication)

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## Introduction

The  $\beta$ -ray activity of radioactive bodies has up till now proved to be a baffling problem. The points at issue are summarised in Gamow's *Constitution of Atomic Nuclei*, pp. 52—54, and in *Radiations from Radioactive Bodies* by Rutherford, Chadwick and Ellis. They are also discussed at some length by Bohr in his *Faraday Lecture* (Feb. 1932). We shall later quote freely from Bohr, but some fundamental difficulties may be pointed out at the outset.

The older view of the constitution of the nucleus was that it should be regarded as composed of  $A$  protons ( $A$ =mass number) and  $A - Z$  electrons ( $Z$ =nuclear charge). A large number of these protons and electrons may exist in the compound form of  $\alpha$  particles ( $4p + 2e$ ) or some other composite structures. But even allowing for these, the existence of a number of free electrons had to be postulated inside the nucleus. On the other hand, the evidence of hyperfine structure, as was first pointed out by de Kronig, definitely proves that the electron cannot exist in the free state in the nucleus, for then the magnetic moment of the nucleus should have the magnitude of the Bohr magneton, while the hyperfine structure of spectral lines definitely shows that the moment has the magnitude of the proton magnet ( $\frac{1}{1836}$  times the Bohr-magneton). At the present time, it is almost universally held that the nucleus consists of  $Z$  protons, and  $A - Z$  neutrons, but it is quite possible that a number of these are combined in the form of  $\alpha$ -particles, deutons, etc. *The nucleus contains no electrons free or bound.*<sup>1,2</sup>

But this conclusion is seemingly at variance with the observed fact that in  $\beta$ -ray disintegrations the nuclei are observed to eject high speed electrons spontaneously. The situation is therefore paradoxical. Bohr puts it as follows:—

"Strictly speaking, we are not even justified in saying that a nucleus contains a definite number of electrons, but only that the negative

electrification is equal to a whole number of units and in this sense, the expulsion of a  $\beta$ -ray from a nucleus may be regarded as the creation of an electron as a mechanical entity."

In a later passage, Bohr describes the other difficulties as follows :—

"As regards this last question, much theoretical interest has recently been aroused by the peculiar features exhibited by the  $\beta$ -ray expulsions. On the one hand, the parent elements have a definite rate of decay, expressed by a simple probability law, just as in the case of the  $\alpha$ -ray disintegrations. On the other hand, the energy liberated in a single  $\beta$ -ray disintegration is found to vary within a wide continuous range, whereas the energy emitted in an  $\alpha$ -ray disintegration, when due account is taken of the accompanying electromagnetic radiation and the mechanical energy conversion, appears to be the same for all atoms of the same element."

To the above remarks of Bohr, the following may be added :—

(a) The  $\beta$ -ray disintegration has been observed not only in the case of heavy elements, but also in the light elements potassium and rubidium (or rather the isotopes  $K^{41}$  and  $Rb^{87}$ ). In the case of  $\beta$ -ray bodies associated with the main groups (U, Th, Ac), the life of  $\beta$ -ray bodies is found to vary from 16 years (RaD) to a few minutes, but the light elements  $K^{41}$  and  $Rb^{87}$  possess lives comparable with those of some long-lived heavy radioactive bodies. The life of  $K^{41}$  has been estimated to be  $7.5 \times 10^{10}$  years that of  $Rb^{87}$  to be  $10^{11}$  years.<sup>3</sup> It is quite possible that there may be a number of  $\beta$ -ray elements possessing longer lives which are still undiscovered, as the activity of such bodies is likely to be extremely feeble, and difficult of detection. In support of our view, we may cite the case of Ac, RaD . . . which were long regarded as undergoing *rayless* changes. They are not actually rayless, but the  $\beta$ -rays are exceedingly feeble, on account of the long life of these bodies.

*From these remarks it will be clear that there is no essential difference between the orders of ranges of the lives of  $\beta$ -ray and  $\alpha$ -ray bodies.*

(b) Ellis<sup>4</sup> has shown in numerous papers that one  $\beta$ -particle is emitted per one disintegrating atom, so that the possibility that the expulsions are due to some external agency seems to be ruled out. They are spontaneous processes like  $\alpha$ -ray disintegration.

(c) The distribution of energy in the  $\beta$ -ray spectrum—This point has formed the subject of investigation by a large number of workers. The curves bear some resemblance to Planck's curve for blackbody radiation but unlike that curve, it has got a limit on the high energy side and the maximum is ill-defined. They also present some similarity to the curves obtained by Kuhlenskampf on the distribution of intensity in the continuous X-ray spectrum.

There has been an idea that the  $\beta$ -rays are probably emitted with quite a definite energy from the nucleus, but in its passage through the outer shell

of electron, it suffers diminution in energy owing to collision or scattering but this view has been disproved by Ellis.<sup>5</sup> Lately, attempts have been made to determine the maximum energy as accurately as possible and to deduce from it a relation similar to that of Geiger and Nuttall for  $\alpha$ -ray bodies.

The latest exponent of this idea is Sargent<sup>6</sup> who found in a recent paper that every  $\beta$ -ray disintegrating atom is distinguished by having a definite end-point in its energy-spectrum. But a reference to his figures shown in Table VI, p. 670, and his curves on p. 671, Fig. 2, shows that there is not much evidence of a relation. For the points lie on three distinct curves, and the radioactive bodies belonging to the same family do not lie on the same curve. Secondly, if the Geiger-Nuttall law for  $\alpha$ -ray bodies is expressed in the form  $\lambda = aE^n$  where  $E$  = energy of the  $\alpha$ -particle varying between 4 to 8 million electron-volts,  $\lambda$  varies from  $10^5 \text{ sec}^{-1}$  (Th C) to  $10^{-18} \text{ sec}^{-1}$  (U),  $n$  is found to vary from 65 to 100. But for the  $\beta$ -ray bodies,  $E$  varies from  $3.5 \cdot 10^4$  evs to  $3.15 \times 10^6$  evs, i.e., a range of about 1 to 100, but  $\lambda$  varies from  $10^{-2} \text{ sec}^{-1}$  to  $10^{-9} \text{ sec}^{-1}$  and if  $\lambda$  be put  $= bE^n$ ,  $n$  varies from 3 to 7. The attempt to trace a causal connection between the decay constant and the maximum  $\beta$ -ray energy does not appear to have been successful. We shall see later that no such causal connection is expected.

*The fact that the  $\beta$ -ray bodies follow the same law of decay as  $\alpha$ -ray bodies can, however, point to only one conclusion, i.e., the phenomenon is due to the leakage of  $\alpha$ -rays through a potential barrier, but somehow the  $\alpha$ -ray does not leave the nucleus, but a  $\nu$ -ray is generated in its place.*

Bohr weighs the probability that the continuous  $\beta$ -ray energy spectrum may be due to differences in the energy contents of the individual parent atoms leading to small and undetectable differences in their mass, but finally decides against this view. The following are his words.

"Unless the expulsion of  $\beta$ -rays from atomic nuclei, contrary to expectation, is not a spontaneous process but caused by some external agency, the application of the principle of energy conservation to  $\beta$ -ray disintegration would accordingly imply that the atoms of any given radio element would have different energy contents. Although the corresponding variations in mass would be far too small to be detected by the present experimental methods, such definite energy differences between the individual atoms would be very difficult to reconcile with other atomic properties. In the first place, we find no analogy to such variations in the domain of non-radioactive elements. In fact, as far as the investigations of nuclear statistics go, the nuclei of any type, which have the same charge and within the limits of experimental accuracy, the same mass, are found to obey definite statistics in the quantum mechanical sense, meaning that such nuclei are not to be regarded as approximately equal, but as essentially identical. This conclusion is the more important for our argument, because in absence of any theory of the intra-nuclear

electrons, the identity under consideration is in no way a consequence of quantum mechanics, like the identity of the extra-nuclear electronic configurations of all atoms of an element in a given stationary state, but represents a new fundamental feature of atomic stability. Secondly, no evidence of an energy variation of the kind in question can be found in the study of the stationary states of the radioactive nuclei involved in the emission of  $\alpha$  and  $\gamma$  rays from members of a radioactive family proceeding or following a  $\beta$ -ray product. *Finally, the definite rate of decay which is a common feature of  $\alpha$ - and  $\beta$ -ray disintegrations points even for a  $\beta$ -ray product, to an essential similarity of all the parent atoms, in spite of the variation of the energy liberated by the expulsion of the  $\beta$ -ray.* In absence of a general consistent theory embracing the relationship between the intrinsic stability of electrons and protons and the existence of the elementary quanta of electricity and action, it is very difficult to arrive at a definite conclusion in this matter."

We have quoted this passage in full, because after this paper was written, we came across a paper by Beck<sup>7</sup> where this idea of hypothetical differences in the energy contents of the individual parent atoms resulting in small and undetectable differences in their mass has been revived to account for the continuous energy distribution amongst the ejected  $\beta$ -rays.

Finally, in order to explain events, Bohr wants to sacrifice the law of conservation of energy and suggests the following process:

"At the present stage of atomic theory, however, we may say that we have no argument, either empirical or theoretical, for upholding the energy principle in the case of  $\beta$ -ray disintegrations, and are even led to complications and difficulties in trying to do so. Of course, a radical departure from this principle would imply strange consequences, in case such a process could be reversed. Indeed if, in a collision process, an electron could attach itself to a nucleus with loss of its mechanical individuality, and subsequently be recreated as a  $\beta$ -ray, we should find that the energy of this  $\beta$ -ray would generally differ from that of the original electron. Still just as the account of those aspects of atomic constitution essential for the explanation of the ordinary physical and chemical properties of matter implies a renunciation of the classical ideal of causality, the features of atomic stability, still deeper-lying, responsible for the existence and the properties of atomic nuclei, may force us to renounce the very idea of energy balance."

The above short summary will probably convey some idea regarding the complexity of the problem.

## 2. Electrofission of Light Quanta

It appears that the  $\beta$ -ray disintegration admits of a rather simple interpretation on the basis of the recent experiments by Anderson and Neddermeyer,<sup>8</sup> Meitner and Hupfeld<sup>9</sup>, Curie and Joliot<sup>10</sup> on the production of pairs

of positrons and electrons by impact of hard  $\gamma$ -rays with atomic nuclei. As the description of this fundamental discovery, which promises to throw a flood of light on nuclear physics, is still scattered over the pages of many scientific journals, we try to give a connected account of it here. Skobelzyn<sup>11</sup> was the first to use vertical Wilson Chambers placed within a horizontal magnetic field for photographing the track of cosmic rays. He found that the cosmic rays gave rise to tracks of  $\beta$ -rays possessing extremely high energy. In some cases, the mass-equivalent of the energy was as great as 50 ~ 100 times the rest-mass of the electron. On repeating these experiments, Anderson<sup>8</sup> found that in addition to the tracks due to high energy  $\beta$ -rays there were others possessing equal curvature, but bent in the opposite direction. From the nature of ionisation along these tracks, it was clear that they were due to particles of the same type as electrons, but possessing an opposite, *i.e.*, a plus charge. To this particle, which is the exact positive analogue of the electron, the name *positron* was given. Subsequently Meitner and Hupfeld<sup>9</sup> obtained similar paired tracks of electrons and positrons by taking Wilson photographs of Be-radiations impinging on Pb and Anderson and Neddermeyer and Curie and Joliot<sup>10</sup> showed that even the hard  $\gamma$ -rays from ThC' having the energy 2.0 *mevs* can give rise to such paired tracks (*mevs* stands for million electron volts).

### How are the pair positron and electron produced?

Anderson and Neddermeyer, and Blackett and Occhialini<sup>11</sup> further showed that this production of "paired ions" accounts for a number of unexplained facts. Gray and Tarrant<sup>12</sup> had previously shown that hard  $\gamma$ -rays show an anomalous absorption which is not accounted for by the Klein-Nishina formula for scattering. The anomalous absorption was found by them to start at the  $\gamma$ -ray energy 2 to 3 *mevs*. Later Gentner<sup>13</sup> fixed the limit at 1.2 *mevs*. We have to remember in this connection that  $m_0c^2$ , the rest energy of the electron corresponds to  $5 \times 10^5$  *ers* and thus the energy of a pair of electron and positron at rest is equivalent to 1 *mevs*. Hence there is a connection between the beginning of anomalous absorption, and the production of "paired ions." Blackett and Occhialini<sup>11</sup> suggested that within the nucleus, the  $\gamma$ -ray is split up, under the intense electric field, into a pair consisting of a positron and an electron. Oppenheimer and Plessett<sup>14</sup> regarded the phenomenon as a photo-electric effect, the  $\gamma$ -ray quantum lifting an electron occupying one of Dirac's negative energy states into a positive energy state, thus simultaneously creating an ordinary electron and a "hole" which according to the ideas of Dirac will correspond to the positron (*vide* § 3). Curie and Joliot<sup>10</sup> have proposed to denote this phenomenon as "*materialisation of quanta*."

Blackett further showed that the hypothesis of the splitting of the quantum inside the nucleus explains another interesting observation by Gray<sup>12</sup> and others. The former has subjected the nuclei of many atoms to hard

$\gamma$ -rays from  $\text{ThC}'$  and found that the nuclei were thereby excited to a fluorescent radiation of approximate wavelengths 12X. units and 24X. units. The first possesses an energy of 1 *mevs* and the second  $\frac{1}{2}$  *mevs*. According to Blackett, though the  $\gamma$ -ray may split up inside the nucleus into a pair of positrons and electrons, but the two may again combine either inside the nucleus or just outside. When they combine inside the nucleus only one quantum of energy 1 *mevs* units may be produced. If they combine outside, two quanta each of energy 0.5 *mevs* units will be produced.

We are of opinion that the phenomenon of conversion of a  $\gamma$ -ray into a pair of ions of opposite sign, confirmed by so many investigators in different parts of the world, should be designated by a more expressive term than Curie and Joliot's *Materialisation of Quanta* and the round-about phraseology about holes, etc., borrowed from Dirac's theory should be avoided, and we have ventured to suggest the term '*Electrofission of Light Quantum*...' which clearly expresses the idea that under the influence of the nuclear fields, the quantum of energy undergoes a '*fission*' into elementary charges of opposite sign, the balance of energy being distributed as kinetic energy amongst the two products in a way which is still to be determined. The possibility of the reverse process of two charges neutralising each other in a direct collision has been postulated by many astrophysicists in a slightly different form. But when these predictions were made, the positive unit of electricity was known to be always associated with the mass in a proton, and nobody could conceive of a positron, hence they always talked of annihilation of proton and electron, but the hypothesis has always lacked vigour on account of want of experimental proof. The process as now actually found is different from the early hypothesis about annihilation in many other points.

### Theoretical Predictions about Positron

It may be added at this stage that grounds for the advent of the positron were to some extent prepared by the predictions of Dirac<sup>15</sup> from his relativistic theory of the electron. In this he was first led to postulate the existence of an elementary particle having the charge- $e$  but possessing the negative energy  $-mc^2$ . Such a particle (the anti-electron) would possess very weird properties which have not been observed. We quote from Gamow.

"For such particles the force and acceleration are directed in opposite directions. If two electrons, one of 'positive' and the other of 'negative' mass, meet then the first will be repelled and the second attracted to the other one; both electrons will fly away one behind the other with infinitely increasing velocity, giving an amusing picture of electronic races."

Later, Dirac developed a theory of 'holes' to account for 'positive charges'. He postulated that in Nature all the negative energy states are usually fully occupied, but sometimes a hole may appear. A positive energy

electron will then jump into the hole, resulting in the neutralisation of charges and release of the energy  $> 2 m_0 c^2$  as radiation in the form of one or two quanta. The process is thus equivalent to the so-called annihilation of charges. The 'hole' can be identified as a "unit positive charge". But it could not be identified with the 'proton' because the mass of the proton is 1836 times heavier than that of the 'hole'. The discovery of the positron exactly corresponds to Dirac's hole, but sweeps away the misleading ideas about particles capable of possessing "negative energy-state". These ideas are not a little responsible for creating confusion in contemporary scientific thought. Instead of an anti-electron with a negative energy we have now a straightforward positive analogue to the electron with positive charge and positive energy.

### The Proton

The question of the nature of the Proton now becomes a problem. According to one view, the proton is not a fundamental particle but is a compound of the neutron and the positron. If this view be correct, the neutron is merely 'mass' possessing an inherent tendency to capture positrons, but behaving in a different way towards electrons which they cannot capture for if this could take place, we could obtain a negative proton. There is also certain amount of experimental evidence in favour of this view. Anderson and Neddermeyer, as well as Curie and Joliot found in their experiments on *Electrofission* of  $\text{ThC}'$   $\gamma$ -ray quantum that more electrons are obtained than positrons, Curie and Joliot<sup>10</sup> give the following figures.

*Number of positrons per 100 electrons (Magnetic field 1100 gauss).*

Al	Cu	Pb	U
5	18	30	40

But working with cosmic rays which can now be definitely taken to be super  $\gamma$ -rays, it has been found by Anderson as well as Kunze<sup>16</sup> that the number of positron tracks is equal to the number of electron tracks. These results, can be explained on the hypothesis that positrons are easily absorbed by the constituents of the nucleus, possibly neutrons, while electrons are repelled by them. Only very high energy positrons can resist capture by nuclei. Further, if the neutron, the electron and the positron are fundamental particles, they should possess the angular momentum  $\frac{1}{2} \frac{h}{2\pi}$  (and be guided by Fermi-Statistics). The protons according to this view may have varying angular momentum depending upon the state of the combination between the neutron and the positron, a view which seems to be in agreement with the latest results of



Stern and Eastermann.<sup>17</sup> According to Chadwick,<sup>18</sup> however, the proton is probably fundamental, and the neutron is a "dipole" composed of the proton and the electron. As the difference of mass on the two views is of the order of '00054, the question cannot probably be ever determined by a precision estimation of masses, but only by investigation of the response of the neutron to light quanta. For, Chadwick's neutron being a dipole, would be highly reactive towards electromagnetic radiation, while the mere 'mass-neutron' is not expected to be reactive. Even on this point, we are not on very sure grounds, for according to one of us, the neutron is a magnetic dipole, composed of two free Dirac's magnetic poles separated by a distance of  $\frac{e^2}{Mc^2}$  which is  $\frac{2}{3}$  times the protonic radius, but these views have no effect on the present course of investigation.

Though not directly connected with the subject-matter of this article, it may be pointed out that the two views regarding the proton will have different consequences in astrophysics. According to many astrophysicists, hydrogen is found in abundance in many stars, and there is a likelihood that the chief constituent of all stellar matter is hydrogen. This must exist in the interior as protons. The proton, if it is a compound will be further broken up into the neutron and the positron, for the binding energy is small, between  $10^4$  to  $10^5$  evs, and even the smallest temperature ascribed to stellar interiors is sufficient for the complete breaking of the proton. The other (Chadwick's) view does not allow this breaking, for the proton being fundamental cannot be further subdivided. So on the first view the stellar core will consist of neutrons, positrons and electrons; while on the second view, it will consist of protons and electrons. This is fraught with far-reaching consequences. For the neutrons have been found to possess the remarkable property of passing through matter till they are stopped by the nucleus, and when they strike the nucleus, they excite radical changes in it, resulting in the emission of protons,  $\gamma$ -rays,  $\alpha$ -particles. One of us is at present engaged in working out a model of a star whose interior is mainly composed of neutrons.

### Annihilation of Charges

In this connection, we may refer to the hypothesis about annihilation of matter advocated by Jeans and Eddington<sup>19</sup> to account for the source of stellar energy. A certain amount of vagueness is always attached to such hypothesis, for annihilation literally means to be reduced to nothing, but the process described here is very different from unalloyed nihilism on the part of fundamental particles, for when an electron and proton hit each other, a neutron and a  $\gamma$ -ray is produced. There is no violation of the principle of

conservation of energy or momentum, so nothing is annihilated except that the charges seemingly disappear. The energy of the  $\gamma$ -ray is available for supplying the stellar energy, but it is not yet known whether the mass of the neutron can be converted into energy. Again, when an electron and positron collide outside a nucleus an application of the principle of conservation of energy and linear momentum shows that two quanta must be produced in their place. If they collide inside the nucleus, there may be one quantum as the nucleus can bear certain amount of the shock and thus ensure the obedience to the law of conservation of momentum. In both these processes, there are more variables than equations, and hence the energy of the quanta cannot be uniquely determined. In none of these processes of collision there is either annihilation of mass, or energy, and not even of charges, for in the quantum formed, the two charges probably retain their individual existence as components of a dipole moving with the velocity of light, and they can again be separated when a "fission" takes place. This picture is very different from what is conveyed by Eddington's picturesque description of the phenomenon as a "joint suicide of the electron and the proton."

### 3. Explanation of $\beta$ -Ray Activity

We shall now discuss how the  $\beta$ -ray activity can be explained. It is clear that if a  $\gamma$ -ray or supergamma (cosmic) ray coming from outside can split up inside the nucleus into an electron and a positron, it will be much more easier for a  $\gamma$ -ray, of sufficient energy, which is produced within the nucleus to undergo spontaneously such a process of electrofission. Of the pair produced, the electron will be ejected as a  $\beta$ -ray, but the positron cannot usually escape, for it will be prevented by the potential barrier from escaping when such barriers exist, or attach itself to some neutron which is present inside the nucleus. For we have already seen that the neutron has an affinity for the positron, but none for the electron. The net charge in any case will be increased by unity, as is observed in  $\beta$ -ray disintegration. It attaches itself to a neutron,  $\gamma$ -rays of small energy of the order of '05 mevs would probably be given off, which are always observed in a  $\beta$ -ray disintegration. It is not difficult to account for the continuous distribution of  $\beta$ -ray energy, for the primary  $\gamma$ -ray while undergoing '*Internal electrofission*' may have its energy divided between the pairs within wide limits and a certain amount of energy will be communicated to the nucleus. But exact mathematical calculations can be carried out only when more data are forthcoming. The problem of annihilation of two charges of opposite sign which is the converse of the present problem has been discussed by Dirac<sup>20</sup> Tamm, and Oppenheimer on the basis of Dirac's holes as positrons.

According to the above view, the  $\beta$ -ray emission is only a secondary process, the primary phenomenon which starts the chain of events which we call a  $\beta$ -ray disintegration is the generation of primary  $\gamma$ -ray within the nucleus. We may now ask ourselves: how is this  $\gamma$ -ray generated? For this, a discussion of the recent theories of a  $\alpha$ -ray disintegration is necessary.

It is now well known that classical mechanics offered no solution to the problems of radioactivity. Gamow, and Gurney and Condon first suggested methods for explaining many features of radioactivity from the standpoint of wave-mechanics. The methods were elaborated in great detail by Gamow who succeeded in achieving a good deal of success in explaining the essential features of  $\alpha$ -ray disintegration and  $\gamma$ -ray origins. Very substantial contributions were also made by Laue, Fowler, Fowler and Wilson, Atkinson and Houtermans, Schrodinger and others.<sup>21</sup>

All these works suffer from the defect that we have as yet no sure knowledge of the structure of nucleus, *i.e.*, of the constituent particles, the statistics obeyed by them and the laws of interaction towards each other. Hence, as in the earlier stages of study of many other branches of science, ad hoc hypotheses based on previous knowledge, have to be invented, and the value of these hypotheses is determined by the amount of success achieved by them. It now seems to be fairly certain as mentioned in the introduction that the nucleus consists of protons and neutrons only, and that there are no free electrons (or negative charge in any form) in the nucleus. Most of the protons are combined in the form of  $\alpha$ -particles. From a scrutiny of Aston's mass-defect curves it has been deduced that elements after Pb are mostly built up by the addition of only  $\alpha$ -particles to the Pb nucleus. Thus U (238/92) the parent of radioactive elements having  $A=4n+2$  consists of a Pb nucleus (206/82) with 8  $\alpha$ -particles about it. Th (232/92) the parent of radioactive elements having  $A=4n$  consists of the lead nucleus (208/82) with 6  $\alpha$ -particles about it. The mass-defect curve shows that the binding force of these  $\alpha$ -particles is very small, *i.e.*, they can be regarded as free to a certain extent. They are prevented from leaving the nucleus by the existence of a potential barrier about the nucleus, whose height is larger than the energy of the  $\alpha$ -particles in the crater. According to classical mechanics it will be impossible for the particles to leave the nucleus, but it was suggested by Gamow, and Gurney and Condon that according to wave-mechanics they can be regarded as waves, and thus possess the property of leaking through the barrier. The rate of leakage through the barrier determines the decay of the elements. Various hypotheses have been postulated regarding the height, size and form of the barrier, but the final results agree in their essential features. There is, however, a large amount of divergence in the methods of mathematisation of the ideas. Laue and others take simplified cases,<sup>21</sup> in which the process is regarded as stationary

and calculate the rate of leakage through an oblong-shaped potential barrier. Though the mathematics is much simplified, the picture does not evidently correspond to facts as the process cannot be regarded as stationary (independent of time). Gamow,<sup>22</sup> on the other hand, introduces complex eigen-values, and by a suitable formulation of boundary conditions, obtains values of decay constants as well as of the eigen-values for the energy of the  $\alpha$ -particles inside the crater. His final results are

$$\log \lambda = \log \frac{h}{4\pi m r_0^2} - \frac{8\pi^2 e^2 (Z-2)}{h V_e M} + \frac{16\pi e m^{\frac{1}{2}} (Z-2)^{\frac{1}{2}}}{h M} r_0^{\frac{1}{2}}$$

$$E = \frac{n^2 h^2}{8\pi m r^2} + U_0 = \frac{1}{2} m V_e^2$$

where  $e$ ,  $h$  and  $Z$  have their usual meaning.  $M$  is the mass of the  $\alpha$ -particle and  $V_e$  is the velocity with which it escapes.  $r_0$  is the "radius" of the product nucleus and  $V_0$  mean potential energy of an  $\alpha$ -particle inside it.

It is seen from the above formula that they involve two constants, *viz.*,  $r_0$  the equivalent radius of the crater, and  $V_e$  the velocity of ejection of the  $\alpha$ -particle. According to our picture,  $r_0$  should not much vary for elements belonging to the same radioactive family while the radius  $r_0$  is found to vary in a regular way from U to RaC and from Th to Th A. We get abnormally low values for it when we come to those interesting products RaC, ThC and AcC which disintegrate in a dual fashion, emitting both  $\alpha$ - and  $\beta$ -rays. The value of  $r_0$  falls from  $8.3 \times 10^{-12}$  for RaA to  $6.3 \times 10^{-12}$  for RaC; and from  $8.1 \times 10^{-12}$  for ThA to  $6.6 \times 10^{-12}$  for ThC.

We revert again to the question as to how the primary  $\gamma$ -ray referred to above which, by undergoing internal electrofission gives rise to the observed  $\beta$ -decay, is generated. It is reasonable to postulate that there are more than one potential barrier inside a nucleus, though their exact nature (*i.e.*, their height and width) and forms can only be determined when we have a sufficient knowledge of the structural arrangement of the particles constituting the nucleus. Our assumption is that the primary  $\gamma$ -ray is generated by the leakage of an  $\alpha$ -particle through an internal potential barrier, *i.e.*, the  $\alpha$ -particle leaks from one crater to another, both within the nucleus. It occupies a lower energy level in the new crater and the balance of energy constitutes the primary  $\gamma$ -ray. This primary  $\gamma$ -ray suffers an electrofission producing a positive and a negative electron. The positive electron attaches itself to one of the neutrons present inside the nucleus, thus raising the nuclear charge by unity. The negative electron is ejected, which constitutes the usual  $\beta$ -ray. The combination of the positron with the neutron will liberate some energy (nearly equal to the difference between the masses of positron+neutron, and the proton) and this may account for the soft  $\gamma$ -rays that usually accompany a

$\beta$ -disintegration. The life of the  $\beta$ -decay is determined by the rate of leakage of the  $\alpha$ -particle from one inside crater to another and hence to the first order will be independent of the energy of the  $\beta$ -rays. Thus no simple relation (unlike the case of  $\alpha$ -decay) is expected to exist between the maximum energy of  $\beta$ -rays and the life of  $\beta$ -decay, a conclusion which is more or less borne out by Sargent's curves.

On the above view it is to be expected that occasionally a positron may not be captured by the neutron, and it may emerge. The presence of positrons associated with the natural  $\beta$ -decay as suggested by Skobelzyn's experiments lends support to the views herein stated.

The explanation of the continuous energy distribution in the  $\beta$ -ray spectrum offers no special difficulties. In our case the energy of the primary  $\gamma$  ray is shared between the positron and the electron, and so the energy of the electron can vary from zero to a maximum ( $h\nu = c + 2m_0c^2$ ). The exact form of the distribution curve can only be calculated when we make additional assumptions regarding the mechanism of interaction. This will be examined on a future occasion.

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# ON THE DETERMINATION OF THE VALUES OF $\gamma$ FOR AIR SATURATED WITH WATER VAPOUR AT VARIOUS TEMPERATURES.

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## Introduction

The value of  $\gamma$  is very important from the thermodynamical point of view, and a precise study of its variation has an important bearing on the molecular structure of gases and is of great help towards a better understanding of their dissociating equilibria. But the available data of high precision on  $\gamma$  is poor excepting for a limited number of gases and organic vapours. There is hardly any data for  $\gamma$  for air which is completely saturated with water vapour. In the present paper the author wants to report some results of his experiments on the determination of  $\gamma$  for air saturated with water vapour which were carried out at various temperatures ranging between about 15°C. and 80°C. The method used was to determine accurately the velocity of sound in saturated air at various temperatures by means of a resonating tube. This was done by setting up stationary waves in the tube by means of a telephone diaphragm, actuated by a source of constant frequency, *viz.*, a valve-maintained tuning fork oscillator, and measuring the internodal distance  $\lambda/2$ . The tube-velocity of sound in saturated air at any particular temperature was then obtained as a product of the wavelength,  $\lambda$ , and the frequency 'N' of the source. We can then obtain the value of  $\gamma$ , from the basic formula  $V = \sqrt{\frac{P \cdot \gamma}{\rho}}$  . . . . (1) if the values of P and  $\rho$  be known.

Recently C. D. Reid<sup>1</sup> has studied the increase in the velocity of sound due to the presence of moisture in air. He determined the velocity of sound at 20°C. in dry air, humid air (45% relative humidity) and in air completely saturated with water vapour (100% relative humidity) and by plotting this increase in the velocity of sound against the percentage relative humidity he has obtained a straight line graph satisfying the empirical relation,  $V_H = V_0 + 0.14 H$  . . . (2) where  $V_H$  is the velocity at any relative humidity at 20°C.;  $V_0$  is the velocity in dry air at 20°C. and H is the relative humidity. The author has also plotted the experimental values for a wide range of

temperature, of  $(V_H - V_O)$ , the excess of the velocity of sound in saturated air over the velocity of sound in dry air at the same temperature, against the moisture content as well as against the temperature. These curves may be seen under section XIII.

## II

### Previous Experimental Methods and Discussion

The method of determining the velocity of sound in gases in resonating tubes is still considered by some to be not free from uncertain errors due to the tube-effects such as the dependence of tube-velocity, on the frequency, on the roughness of the wall surface, on its thickness and on the nature of the material of the tube, etc. But through the efforts of many observers in the recent time, specially G. W. C. Kaye<sup>2</sup> and G. G. Sherratt, who made precision measurements of the velocity of sound in different gases, contained in resonating tubes of different diameters and material, many of the uncertain tube-corrections have been totally removed, and for all practical purposes the validity of the Helmholtz-Kirchhoff expression has been firmly established. The divergence of results in connection with the tube-correction is mostly due to inaccurate and inconsistent experiments for which the theory cannot be blamed. This point is discussed fully later on.

On the other hand, the method of determining the velocity of sound in gases depending upon what is sometimes called Pierce's acoustical interferometer, making use of ultrasonic waves (generated either by the quartz crystal oscillator or by the magnetostriction oscillator) is free from all difficulties of tube-correction, on account of very high frequencies used, but involves other uncertain and irregular effects arising out of the diffraction phenomena (and impedance effect) which cause the velocity of sound to be higher than the accepted free-space value; the effect being considerable in case the measurements of wavelength are made when the source and the reflector are not very far from each other or when the frequency used is near about 42 kilocycles<sup>3</sup> per second. Further, the smallness of wavelengths measured (for instance at 60,500 cycles per second, the wavelength in air is only about 0.57 cm.) demand an extreme sharpness of maxima (or minima). But such sharpness of maxima, in practice, cannot be attained for obvious reasons that the positions of the maxima are ascertained from the deflection of the micro-ammeter needle, which even in the vicinity of a reaction, are not very steep. This point, however, may be illustrated from the careful experiments of Martin Grabau<sup>4</sup> who states that the accuracy of the setting of the mean position of a maximum is about 0.5 mm. for a frequency 19,790 cycles per

second (wavelength about 1.75 cms). Thus the ratio of the accuracy of setting to the wavelength observed is  $\frac{0.5}{17.5}$  which is  $\frac{1}{35}$ . While in the present experiments (with the resonating tube) to be described, the accuracy of the setting of the mean position of a maximum was within 0.5 mm. for a wavelength about 350 mms. and hence the ratio of the accuracy of setting to the wavelength is  $\frac{1}{700}$ . This may further be stressed by quoting the divergence of results which W. H. Pielemeier,<sup>5</sup> working carefully with a Pierce acoustical interferometer, obtained in the case of dry air. His values of the velocity of sound in dry air at 0°C. are as follows: 333.3 m. per sec., 332.8 m. per sec., 334.5 m. per sec., 333.8 m. per sec. and 333.7 m. per sec., which differ from each other to such an extent as to be practically of little use if one wishes to calculate the precise value of  $\gamma$  for dry air. These considerations\* led the author to adopt the resonating tube-method for determining the internodal distances with a constant frequency source of sound, and it will be sufficiently proved in subsequent sections of this paper that it is possible to obtain the accurate values of the velocity of sound by the above method.

### III

#### Description of the Apparatus

The apparatus consisted mainly of a movable source of sound mounted at an end of a brass rod which could move it throughout the entire length of the resonating tube; and a fixed reflector. No listening side-tube† was used but instead a microphone was placed just beyond the fixed reflector which was a thin disc of mica. A section of the main portion of the apparatus is shown in Fig. 1. AB is a straight pyrex glass tube (specially ordered for

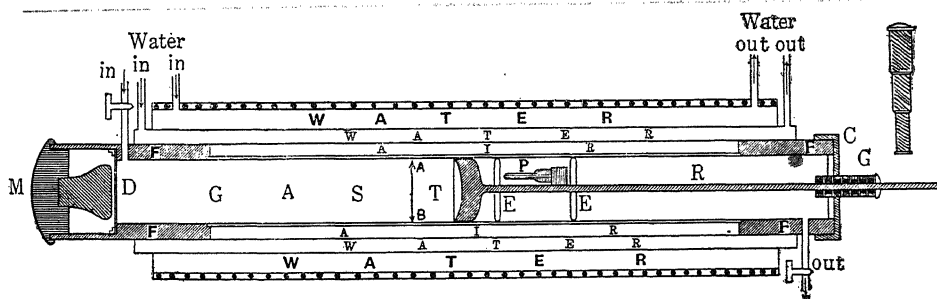


Fig. 1

these experiments) which is about 170 cms. long and 2 mms. thick with a nearly uniform bore of about 6.08 cms. diameter. FF are the brass flanges

\* Moreover, it is very difficult to maintain the oscillations of the quartz crystal in the presence of the large quantities of moisture contained in the saturated air and hence the accuracy of results is impaired.

† See discussion by Mr. D. A. Oliver, Proc. Phys. Soc., Vol. 43; 252; 1931.



fixed at each end of the resonating tube AB. Each flange in turn carries a side-tube of about 3 mms. diameter to serve as an inlet and an outlet for the gas under observation. D is a mica disc about 0.2 mm. thick which is mounted perpendicular to the axis of the tube by means of an arrangement of threaded brass rings which keep the mica disc rigidly fixed. The mica membrane D thus closes one end of the resonating tube and, as noticed before, serves the purpose of a reflector. This end of the tube, 'the reflector end,' to call it so, was made air-tight by means of plaster of paris. The other end of the resonating tube, the telephone end, is closed by means of a brass cap C at the extreme mouth of the flange F. In the centre of this cap there is an air-tight annular gland through which the brass rod R can be moved to and fro without any fear of leakage. The construction of the annular gland G is shown in Fig. 1 which is more or less like the flange arrangement used in piezometers of the usual type. The brass cap C at the end of the tube is also fitted with six terminals insulated from each other by means of mica and ebonite washers. Plaster of paris was used to make this end of the tube also air-tight, as far as possible. A telephone having a resistance of about 2000 ohms, and of such a size as to fit in the tube correctly, was mounted on the brass rod R so rigidly that there was no danger of any relative motion between the rod and the telephone diaphragm. Particular care was taken to keep the telephone diaphragm, perpendicular to the axis of the tube so that the reflector and the diaphragm of the telephone were always parallel to each other. In order to completely avoid the annular space left between the rim of the moving telephone and the wall of the tube, we wrapped round the rim a thin strip of flannel which acted like an elastic soft pad, so that this piston was a good fit in the resonating tube. The pad also served the purpose of eliminating all the undesirable creaking noises produced by the metallic telephone rim of the tube. To allow a more or less free passage of air past the rim of the telephone three small grooves were cut in the pad round the rim. Behind the telephone a perforated ebonite disc E of almost the same diameter as the telephone was mounted on the brass rod, which passed through its centre so rigidly that it moved along with the telephone throughout the tube. Immediately behind the ebonite disc E a platinum thermometer of length about 18 cms. and weighing about 105 grams was mounted by means of brass screws on the brass rod itself, and about 3.5 cms. away from the head of the platinum thermometer another perforated ebonite disc E was fastened rigidly on to the brass rod. Just beyond the mica reflector end of the resonating tube a small Lapel<sup>6</sup> microphone which was mounted on a circular wooden bed was fitted in such a way that the face of the microphone was about a centimetre away from the mica membrane. The circular wooden bed of the microphone fitted tightly in the brass flange F and thus kept the microphone fixed at that particular position. To match with this microphone

an audio-frequency transformer was used. Any sound received through the microphone was directly heard in the head-phones which were connected to the secondary of the transformer. The apparatus was placed on a V-shaped wooden platform.

#### IV

##### Sound Production and Remarks on the Constancy of the Frequency of the Source

The source of sound used in these experiments for setting up stationary waves inside the resonating tube was the telephone T electrically connected to the output terminals of a valve-maintained tuning fork oscillator such as that described by D. Dye.<sup>7</sup> The tuning fork used in the oscillator was made of 'elinvar' steel (Valentine and Carr) with a nominal frequency of about 1000 cycles per second. It was mounted very solidly in a solid block of brass which in turn was very rigidly riveted to a solid rectangular brass plate and the whole arrangement with the electrical attachments was placed in a small wooden box having a thick layer of soft woollen pad at its bottom. The same valve (Phillips A. 415) was used throughout all these experiments.

After making some preliminary experiments with sounds of different intensities in the resonating tube, we found it best to keep the anode voltage at 60 volts; the anode current in this case being of the order of 1.2 milliamperes. Voltages higher than this were deliberately not used in order to avoid sound waves of great intensity in the tube. To maintain the frequency constant, as far as possible, the oscillator was left completely undisturbed so that there was no danger of any change of frequency due to the causes, namely—(1) variation of filament current and anode voltage, (2) variation of grid and anode condensers, (3) variation of the polarising magnetic field, (4) variation of energy taken from the output winding, and (5) variation due to adding a mass to various parts of the mounting or to tilting of the fork.

The only cause of variation of frequency was the temperature of room which varied roughly between 28° C. and 36° C. But since the tuning-fork used is made of elinvar steel for which the temperature coefficient of frequency change is known to be very small, the change in the frequency for a range 28° C. to 36° C. may amount only to about 0.8 parts in ten thousands which being well within the experimental error was completely neglected. The frequency of the note under the actual conditions of the experiments (velocity experiments) as heard in the head phones was determined by comparing this with a certified tuning-fork also of elinvar steel (Valentine and Carr) having a frequency 1000 cycles per second when mounted solidly, as directed in the certificate. Beats were counted and the time was taken by means of a stop watch. This part of the experiment was extremely difficult because of the

fact that the beats counted were very nearly six per second and hence extreme care had to be taken for accurate counting. However, many independent readings by different persons were taken to exactly fix the number of beats per second and the results of all such readings were found to be between 5.9 and 6.0 beats per second, so that the frequency of the note in the resonating tube under identical conditions of the actual velocity experiments comes to be  $(1000-6) = 994$  cycles per second. To test this point further and to facilitate the counting of beats accurately enough for our experimental purposes we loaded the prongs of the certified tuning-fork with equal quantities of soft wax placed symmetrically on both the prongs. The quantities of wax were at first so adjusted that absolutely no beat was observed. After this the quantities of wax on the prongs were halved and the beats produced under this condition were carefully counted. The mean of several such readings, for one experiment, was found to be 3.02 beats per second. Assuming a linear variation of beats with the added masses (wax) to the prongs (a fact approximately true for such a short range as this) we could conclude that the frequency of note in the resonating tube was  $(1000-3 \times 2) = 994$  cycles per second, with a probable error of 0.1 per cent. It may be remarked here that the response of the ear to various frequencies is not the same. It is maximum near 1024—1028 cycles per second so that the choice of this frequency 994 was of great value in obtaining a high degree of accuracy in locating the points of maximum intensity of sound in the resonating tube. Another advantage in the selection of this frequency 994 is that the wavelength (in air about 35 cms.) is about 12 times the radius of the resonating tube (3.04 cms). Lord Rayleigh<sup>8</sup> pointed out that the waves set up in a cylindrical tube will ultimately become plane, provided the ratio of wavelength to the radius of the tube is greater than about 3.4. Sherratt and Awbery<sup>9</sup> have also experimentally found out this ratio to be 4.5 which is a little higher than the theoretical one (3.4). This ratio 12 in our experiments is thus far higher than even the experimental value 4.5 and hence all possibilities of the sound pattern in the resonating tube getting complicated by virtue of its natural transverse vibrations are eliminated so that there cannot be introduced any uncertain error due to this alone in the measurement of the internodal distances.

## V

### Temperature Control and Measurement

Since the temperature coefficient of change in the velocity of sound in air is about 0.6 metres per second, *i.e.*, it is of the order of 1 in 600, it is necessary that the temperature should be controlled and measured with an error not exceeding about 0.5°C. in order to attain a probable accuracy

of about 1 in 1000 in the measurement of the velocity of sound. Naturally in the construction of the apparatus we paid a great attention to those parts of the apparatus which controlled and measured the temperature. To achieve this, a double jacketed cylindrical vessel surrounding the entire length of the tube as shown in figure 1, was constructed. This vessel then was wrapped up in asbestos sheets and nichrome wire was wound round it to heat it electrically. In winding the wire care was taken to keep the spacing as far uniform as possible except at both the ends where the wire turns were a little denser than at the central portion of the vessel. The vessel was again wrapped up in thick sheets of asbestos so that the wire turns lay in between parallel layers of asbestos sheets. A set of variable resistances and an ammeter were placed in series to vary the current in the furnace according to need. In this arrangement the heating or cooling of the gas enclosed in the resonating tube was effected very gradually. This arrangement thus ensured uniformity and accurate regulation of temperature throughout the whole duration of the experiment (about three hours). But we had to wait long before the temperature became steady, in fact we had to wait for eight to ten hours; but once it became steady the outside fluctuation of temperature had very little effect on it.

Still there were three regions in the apparatus which lost heat through radiation and conduction. These were (1) the microphone end of the tube and a little portion, within two cms., of the brass flange which was not enclosed in the heating vessel, (2) the end G of the tube could not be enclosed in the heating vessel, and (3) the brass rod itself which, of necessity must make to and fro motion while the wavelength measurements were being made. The first two sources of heat loss were partly stopped by wrapping heavily the uncovered portions with cotton-wool (not shown in Fig. 1). The microphone and its wooden frame served very well to stop the heat flow through that end. The third source of heat loss for practical purposes was not serious as may be ascertained from the readings in table (1). Temperatures lower than the room temperature were obtained and maintained constant by passing a slow current of cold water through the two jackets of the surrounding vessel. The supply of this cold water was obtained from a small cistern kept about five feet higher than the level of the vessel. We made attempts to keep the temperature of the flowing water constant during the experiment, but the arrangement was not so satisfactory as in the case of heating. Temperatures were measured by means of a platinum resistance thermometer. Pure platinum wire of 0.15 mm. diameter was used. The wires were welded by electricity. Resistances were measured on a calibrated Callendar and Griffith's bridge. The sensitivity of the galvanometer used was  $10^{-8}$  amperes per mm. deflection at a distance of one metre. Preliminary experiments were performed to determine the value of  $\delta$  for the particular wire used.

The determination of the sulphur boiling point was however not very accurate. The mean value of  $\delta$  found was 1.53. All precautions were taken to determine the fundamental interval accurately. The temperature with this arrangement could thus be ascertained accurately to about  $0.05^\circ\text{C}$ . To check the thermometer readings, we calibrated the platinum thermometer by directly comparing it with an accurate mercury thermometer reading up to  $\frac{1}{10}$  of a degree C.

While taking observations for the wavelength determination, the readings of the platinum thermometer also were taken at each maximum. A typical set of readings is given below :—

*Table 1*  
Room temperature  $32^\circ\text{C}$ .

Order of maxima.		Resistance of pt. ther. in ohms.	Temp. in degrees C.
1st max.	...	3.0702	51.4
2nd "	...	3.0703	51.4
3rd "	...	3.0704	51.4
4th "	...	3.0703	51.4
5th "	...	3.0704	51.4

## VI

### The Measurement of the Distances between Successive Maxima

For the precise measurement of half wavelength it is clear that the distance between the successive maxima should be very accurately measured. An error of  $\pm 0.05$  cms. for  $N=1000$ , (half wavelength about 18 cms.) may vitiate the result by as much as 1 metre in the velocity and much more in the cases of higher frequencies. In order to avoid this source of error we measured the length by the comparator method making use of a travelling microscope which was very rigidly fixed on the same wooden V-bench on which the main apparatus was fixed. Great care was taken to avoid any relative motion between the microscope and the resonating tube by clamping both the tube and the microscope very rigidly. The microscope was clamped in such a position that it could be focussed on the brass rod very easily. Since the rod moved through grooves on wooden supports it always remained perfectly horizontal and so always in sharp focus for all positions of the travelling microscope. Very fine cross ( $\times$ ) scratches at intervals of about  $10^6$  cms. were made on the entire length of the brass rod in

such a way that they were all in a straight line on it. The actual distances between the scratches were measured by the same travelling microscope. They are set down in table (2) below:—

*Table 2*  
Room temperature 32°C.

Distances between scratches on rod.			Cms.	Distances between scratches on rod.			Cms.
1st and 2nd	...	...	11.06	7th and 8th	...	...	10.02
2nd and 3rd	...	...	10.03	8th and 9th	...	...	10.07
3rd and 4th	...	...	10.05	9th and 10th	...	...	10.00
4th and 5th	...	...	10.09	10th and 11th	...	...	10.02
5th and 6th	...	...	10.01	11th and 12th	...	...	10.03
6th and 7th	...	...	10.00				

The temperature coefficient of the linear expansion for brass is about  $18.9 \times 10^{-6}$  cms. and so the increase in length per degree between any two scratches (10 cms. length) would be  $18.9 \times 10^{-5}$  cms. Since that portion of the brass rod which was under the microscope was exposed to the outside atmosphere it was comparatively cooler than the resonating tube and hence its temperature did not much differ from that of the room. Therefore the expansion in length between any two scratches on the rod would, in an extreme case, amount to only about  $4 \times 10^{-3}$  cms. and hence it was completely neglected. The effect of the sagging of the brass rod was also found to be quite negligible. This was studied by direct measurement, by placing a calibrated steel scale lengthwise on the glass tube, (the furnace having been removed) and taking readings on the travelling microscope scale as well as on the steel scale simultaneously. The scale of the travelling microscope was calibrated by comparing it with a standard platinum-iridium scale kept for this purpose in the laboratory. The procedure of the measurement of the half wavelengths was as follows:—

A node was located by a proper adjustment of the position of the telephone inside the resonating tube and the microscope moved to one of the convenient scratches on the brass rod, which lay in the range of the travelling microscope scale and the scale reading was noted down, say, the second scratch on the brass rod coincided with the cross wires of the microscope when its position on the scale was 4.03 cms. Then the rod R was slowly pulled out and the second node was located. The microscope was then moved to some other convenient scratch and the scale reading taken, say, the third scratch coincides with the microscope reading 11.56 cms. From these readings we immediately get the distance between the first node and the second node as  $(11.56 - 4.03) + 10.03$ ,

the distance between the second and the third scratch on the rod (table 2), which is equal to 17.56 cms. The same process was carried out for the rest of the nodes.

## VII

### The Location of Maxima

The points of maximum sound intensity, that is the nodes, being quite sharp, were easily detected. This was done, as follows, in two ways:—

(1) The brass rod carrying the telephone was moved slowly to a point of maximum intensity of sound which was audible only for a short range of

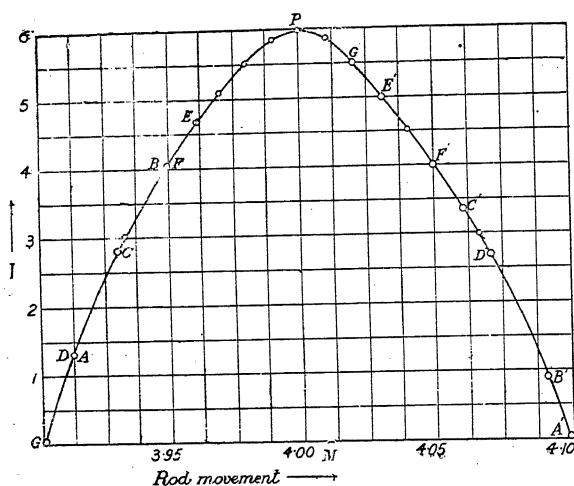


Fig. 2

the movement of the rod R (about 0.5 cm.) and two points were carefully marked, one while approaching the maximum and the other while receding from it, and the corresponding readings for these two points were taken on the travelling microscope scale. The mean of these two readings was then taken to indicate the correct position of the maximum on the assumption that the curve (Fig. 2), showing in an arbitrary way, the variation

of intensity with distances of the rod movement, is symmetrical with respect to the perpendicular P.M. The difference between these two readings was seldom greater than about 1.5 mms. and was usually of the order of 1 mm. Several such pairs of readings were taken to locate a maximum. This process was repeated for every maximum. The whole process was then repeated by moving the rod in the opposite direction and the readings were obtained. For brevity this complete process may be called a run. To ascertain the consistency in the exact position of the maxima, several such runs were made, and the mean of all taken to calculate the final results.

These experiments were mostly carried out in perfect silence during the night with all the doors of the room closed. This not only facilitated the location of maxima, but also helped a good deal in keeping the temperature constant to a better degree than in the day-time when the air draughts disturbed it slightly.

(2) The second method of locating maxima was to attempt to locate the point P (Fig. 2) itself directly by a method of oscillation. This method requires a good deal of experience and practice and so is more

difficult than the first one, but all the same, it is more precise than (1). The difference of the reading (c) among themselves was of an order of 0.5 mm. The mean of ten such readings when compared with the mean of five pairs of readings, taken as described under (1) differed from each other. The order of difference was about 0.2 mm. But when the difference was found greater than this we placed more weight to the readings under (2) than those under (1). The following arbitrary curve (Fig. 2) which approximately represents the intensity of sound (as deduced from impression left on the memory) as the brass rod is moved in the vicinity of a maximum from one end to the other end situated symmetrically opposite to it, shows the accuracy of setting as well as the relative merits of methods (1) and (2) discussed above. The ends here do not mean exactly the limits between which the sound is audible; which in the case cited above would be about 5 or 6 mms. but by this we mean that we have to select two points such as A and A' of nearly equal intensity on either side of P and retain them in memory such that when the observations are repeated they may be located easily. These facts are illustrated from actual readings and may be considered as a typical example to clarify the principle of the method graphically, the readings below refer to the position of a maximum when the corresponding position of the second scratch on the brass rod, as read by the travelling microscope, was that given in table (3) below. The readings under (a) are the readings obtained while approaching the maximum and those under (b) taken while receding from it, and (c) are the readings obtained as described under method (2).

Table 3

Points.	Readings (a) in cms.	Points.	Readings (b) in cms.	Mean of (a) and (b) in cms.	Readings (c) in cms.
A	3.91	A'	4.10	4.005	4.02
B	3.95	B'	4.09	4.020	4.00
C	3.93	C'	4.06	3.995	3.98
D	3.91	D'	4.07	3.990	4.01
E	3.96	E'	4.03	3.995	4.02
F	3.95	F'	4.05	4.000	4.00
G	3.90	G'	4.02	3.960	3.99
					3.98
					3.99
					3.97
					3.98
					4.01
					4.02
					4.01
					4.00
				mean=3.999	mean=3.991



From the curve (Fig. 2) the superiority of method (2) to method (1) is obvious, for it is likely to judge all points in (a) group as lying between, say, G and C and all points in the (b) group as lying between, say, P and F, thus tending to shift the mean value, P, to some such point as E. The error might become serious if the difference between the readings in an individual pair, be of the order of about 4 or 5 mms.

### VIII

#### **Filling the Resonating Tube with Air completely Saturated with Water Vapour**

At first we tried this part of the experiment by simply blowing air through four wash bottles containing water and passing this air through the resonating tube for a few hours. The temperature of the fourth wash bottle, nearest to the gas inlet of the resonating tube was kept about five or six degrees higher than the actual temperature of the resonating tube, so that the final temperature of the saturated air, entering the resonating tube, was about the same as that of the air inside the resonating tube itself. In order to avoid the actual water particles entering the resonating tube, we placed wire meshes at several places in the path between the fourth wash bottle and the gas inlet. To ensure, that the air inside the resonating tube was at that temperature completely saturated with water vapour, some preliminary experiments were made. For this purpose, two special platinum thermometers, nearly identical to each other, were screwed, side by side, to the brass rod, R, and inserted in the resonating tube and the temperature of the air inside was thus read simultaneously by both the thermometers. Having done this, the bulb of one of the thermometers was covered with a piece of linen and a small glass tube, bent in nearly a semi-circular shape and closed at one end, to carry a little quantity of water, in order to keep the bulb of the thermometer wet, was fastened to the brass rod. The readings of the wet thermometer were found to be less than those of the dry thermometer by about one or two degrees, a fact which convincingly proved that the air thus saturated with water vapour by merely bubbling it through wash bottles containing water was not at all completely saturated. But at the same time, it was observed that the temperature of the wet thermometer was slowly rising, as time elapsed, so much so that after about an hour there was practically no difference in the readings of the wet and the dry thermometers. This showed us that the presence of water in the attached curved-tube and the wick of the thermometer, in a closed space like this, could slowly saturate the air completely in about less than an hour. It may be mentioned here that by quickly moving the brass rod, the temperature of the wet thermometer fell more than what it did when the brass rod was either stationary or very slowly

moved. In taking the wet and dry bulb readings the quick motion of the rod was avoided, so as to keep consistency with the usual convention of the dry and wet bulb hygrometry.<sup>10</sup> The importance of the necessity of a complete saturation of air in these experiments may be better realized by the actual experimental results given below.—

(a) The air was saturated by simply bubbling it through water and no water was introduced in the resonating tube. Under those conditions the velocity of sound in tube at  $37.5^{\circ}\text{C}$ . was found to be 355.54 m. per second.

(b) A little quantity of water was introduced at both the ends of the resonating tube and the readings were taken after about 15 minutes. The velocity of sound in this case was 356.28 m. per second.

(c) The third set of observations was taken after waiting for some time. The velocity in this case was 356.44 m. per second.

(d) The fourth set of observations was taken after a few minutes more. The velocity found was 356.43 m. per second.

(e) This set of observation was taken after waiting half an hour more. The velocity found was 356.38 m. per second.

These results show the process of saturation adopted in these experiments to be quite satisfactory. The process of saturating the air by mixing it thoroughly with steam and then filling the tube with the mixture was deliberately avoided for fear of the actual particles of water, in suspension in air, getting inside the resonating tube and creating other disturbances not desired.

## IX

### A Typical Set of Observations and Results

The temperature having been regulated and controlled, the resonating tube filled with the saturated air, as described, the internodal distances were located by hearing in the head phones and judging the points of maximum intensity of sound mostly by method (2) described under section VII. The observations were restricted only to the central portion of the tube about 100 cms. and a margin of about 35 cms. tube-length was left at each end of the tube. The arbitrarily chosen first maximum in table (4) was really the third node when counted from the mica membrane. The length of the tube was only 170 cms. and the internodal distance at about  $75^{\circ}\text{C}$ . was somewhat about 20 cms. and so only 5 maxima, at the most, could be located.

The pressure inside the resonating tube during the experiments was always kept equal to the atmospheric pressure which, however, was not constant, but the fluctuations in the atmospheric pressure, during a period of about three to four hours, were usually of an order of about two to four

mms. mercury column. Therefore to get a mean value of the pressure it was read on a Fortin's barometer at least three times during a run which, as noticed before, lasted three to four hours. The mean of the three readings of the barometer was then taken to indicate the true pressure inside the resonating tube for any particular experiment.

The observations of a typical experiment are set down in table 4 (p. 283).

From the same set of observations in table (4) the velocity of sound was calculated in three different ways as follows :—

Method (A): From table (4) and table (2), the distance between the 1st and the 3rd maximum

$$= [9'180 - 3'504] + [30'17, \text{ which is the distance between the 2nd scratch and the 5th scratch}]$$

$$= 5'676 + 30'170 = 35'846 \text{ cms.} \quad \dots \dots \dots (1)$$

In the same way the distance between the 2nd and the 4th maximum

$$= [\text{the distance between the 3rd and the 7th scratch, which is } 40'150] - [11'303 - 7'088]$$

$$= 35'925 \text{ cms.} \quad \dots \dots \dots (2)$$

Likewise the distance between the 3rd and the 5th maximum = [the distance between the 5th and the 9th scratch which is 40'150 cms]

$$= 35'942 \text{ cms.} \quad \dots \dots \dots (3)$$

From these three values of  $\lambda$ , the mean wavelength is 35'907 cms.

The frequency of the source of sound = 994 cycles per sec.

$\therefore$  the velocity of sound in tube in saturated air at 37'5°C. is 356'92 m. per sec.

Method (B): From table (4) and table (2), the distance between the 1st and the 5th maximum as before = (5'016 - 3'504) + 70'200 and hence  $\lambda = 35'856$  cms. and the velocity in saturated air in tube at 37'5°C. = 356'41 m. per sec.

Method (C): The calculations of the velocity of sound by this method may be better realised by a glance at table (5) given below, which, in addition to other facts, shows that the experimental values of the internodal distances are never exactly equal to each other. This may be at first thought of as due to the error of observation itself, but the consistency of results, all in thorough agreement with the statement made above tends to show that this may be due to some real complexities other than the error of observation. In calculating the velocity of sound by method (C) these facts are considered by giving equal weight to all the observations and taking the mean value thus obtained to represent the true value of the velocity of sound in tube. The values

Table 4

1st maximum 2nd scratch at			2nd maximum 3rd scratch at			3rd maximum 5th scratch at			4th maximum 7th scratch at			5th maximum 9th scratch at			Order of max.	Temp. deg. C	
Readings (a) cms.	Readings (b) cms.	Readings (c) cms.	Readings (a) cms.	Readings (b) cms.	Readings (c) cms.	Readings (a) cms.	Readings (b) cms.	Readings (c) cms.	Readings (a) cms.	Readings (b) cms.	Readings (c) cms.	Readings (a) cms.	Readings (b) cms.	Readings (c) cms.			
345	356	349	11.22	11.34	11.28	9.23	9.14	9.18	7.14	7.04	7.07	5.10	4.95	5.00	1st max.	37.5	
348	353	350	11.23	11.39	11.32	9.24	9.12	9.16	7.12	7.02	7.10	5.06	4.94	5.03			
343	354	352	11.26	11.38	11.30	9.22	9.13	9.18	7.15	7.05	7.09	5.07	4.96	5.01	2nd max.	37.5	
346	352	350	11.24	11.35	11.29	9.24	9.15	9.18	7.12	7.04	7.08	5.08	4.93	5.01			
343	353	349	11.25	11.36	11.30	9.25	9.12	9.17	7.14	7.03	7.10	5.05	4.95	5.02	3rd max.	37.5	
346	355	351	11.26	11.33	11.31	9.22	9.14	9.18	7.13	7.02	7.10	5.08	4.98	5.04			
342	357	352	11.24	11.35	11.32	9.24	9.13	9.18	7.13	7.04	7.09	5.07	4.97	5.03	4th max.	37.5	
344	354	349	11.23	11.36	11.30	9.23	9.11	9.16	7.14	7.03	7.07	5.06	4.98	5.04			
347	355	348	...	...	11.30	9.21	9.14	9.17	...	...	7.08	5.08	4.97	5.00	5th max.	37.5	
345	354	350	...	...	11.31	9.23	9.12	9.18	...	...	7.10	...	...	5.02			
345	357	352	...	...	...	...	...	...	...	...	...	...	...	5.03	5th max.	37.5	
348	358	351	...	...	...	...	...	...	...	...	...	...	...	5.01			
...	...	349	...	...	...	...	...	...	...	...	...	...	...	5.00	5th max.	37.5	
...	...	351	...	...	...	...	...	...	...	...	...	...	...	5.01			
...	...	352	...	...	...	...	...	...	...	...	...	...	...	5.02	5th max.	37.5	
...	...	352	...	...	...	...	...	...	...	...	...	...	...	5.00			
...	...	351	...	...	...	...	...	...	...	...	...	...	...	5.00	5th max.	37.5	
...	...	351	...	...	...	...	...	...	...	...	...	...	...	5.00			
mean of $\frac{a+b}{2}$	3501	mean of of (c)	3504	mean of $\frac{a+b}{2}$	11.299	mean of of (c)	11.303	mean of $\frac{a+b}{2}$	9.180	mean of of (c)	9.174	mean of $\frac{a+b}{2}$	7.084	mean of of (c)	7.088	mean of $\frac{a+b}{2}$	5.010
...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	mean of of (c)	5.016

given in table (5) are taken from the same set of observations contained in table (4)

Table 5

The distance between	Cms.	$\lambda/2$ in each case.	V in each case m/sec.	Percentage difference in V from the mean in each case.
1st and 2nd max. ...	17·829	17·829	354·44	-0·560
1st and 3rd " ...	35·796	17·898	355·81	-0·168
1st and 4th " ...	53·710	17·903	355·91	-0·140
1st and 5th " ...	71·712	17·928	356·41	-0·000
2nd and 3rd " ...	17·967	17·967	357·19	+0·224
2nd and 4th " ...	35·881	17·941	356·67	+0·056
2nd and 5th " ...	53·883	17·961	357·07	+0·168
3rd and 4th " ...	17·914	17·914	356·13	-0·084
3rd and 5th " ...	35·916	17·958	357·01	+0·168
4th and 5th " ...	18·002	18·002	357·88	+0·392
	mean	17·930	356·45	

Thus we see that the velocity of sound as calculated from the same set of observations but by different methods differ from each other, namely by methods

- (a)  $V = 356·92$  m/sec.
- (b)  $V = 356·41$  m/sec.
- (c)  $V = 356·45$  m/sec.

The final results of the velocity of sound given in table (6) and Fig. (3) have been calculated by method (C). The differences between the results calculated by (b) or (c) were negligible but those between the results calculated by (a) and (c) were of the order of 0·5m/sec. The accuracy of the results is claimed to be of the order of about 1 in 1000 up to the temperature about 60°C. The accuracy\* of results above this temperature may be somewhat about 5 parts in 1000.

\* The accuracy of results above 60°C. is a little less because at such temperatures the maxima were not sharp, probably due to the large absorption of sound by the saturated air, which, for instance, at 60°C. contains about 130 grams of moisture per cubic metre.

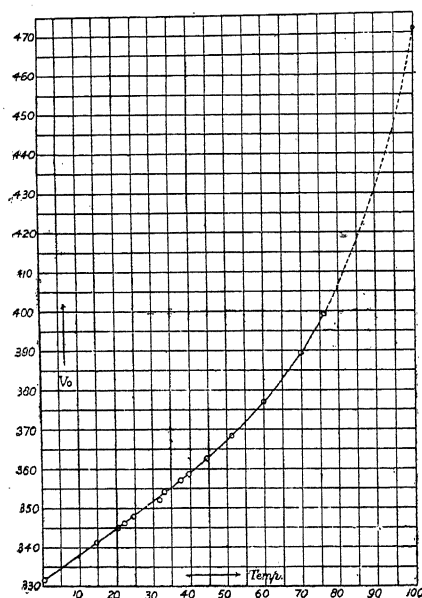


Fig. 3

Table 6

Temperature.	V m/sec.	Tube* correction.	$V_0$ m/sec.
14.9°C	340.87	0.52	341.39
20.3°C	344.50	0.53	345.03
21.9°C	345.54	0.53	346.07
24.6°C	347.36	0.54	347.90
31.7°C	352.29	0.55	352.84
33.1°C	353.45	0.55	354.00
37.5°C	356.49	0.56	357.05
39.5°C	358.09	0.56	358.65
44.5°C	362.03	0.57	362.60
51.4°C	367.82	0.58	368.40
60.1°C	376.63	0.59	377.22
69.7°C	388.77	0.60	389.37
75.6	399.03	0.61	399.64
(100°C)†			(471.5)

## X

## The Tube Correction

Since the tube we have been using in our experiments has quite a smooth surface, we calculated the tube-correction by assuming the experimental value of Kirchhoff constant  $C$  to be correct for all practical purposes. Kaye and Sherratt,<sup>2</sup> after careful and extensive experimentation, of a high order of accuracy, have concluded "that in general the Helmholtz-Kirchhoff equation is substantially valid for smooth tubes." Grüneisen and Merkel<sup>11</sup> who also agree with the form of the Helmholtz-Kirchhoff equation have obtained a value of  $C$  which is in accord with the value of  $C$  obtained by Kaye and Sherratt. These facts may be considered as sufficient to establish the validity of the much disputed expression

$$V = V_0 \left\{ 1 - \frac{C}{2r(\pi N)^{\frac{1}{2}}} \right\} \dots \dots \dots (3)$$

The mean values of  $C$  given by Kaye and Sherratt<sup>2</sup> for the case when dry air was used in glass tubes of smooth surface are 0.51 at 18°C. and 0.61 at 100°C.; the theoretical values of  $C$  for the same two temperatures being 0.56 and 0.69 respectively. The temperature range of our experiments extended from about 15°C. to about 80°C. Therefore in the present series of experiments there are also temperatures other than these two, namely, 18°C. and 100°C., thus rendering it necessary to know the values of  $C$  at these intermediate temperatures also. But the data for the experimental values of  $C$  at different temperatures ranging between 15°C. and 80°C. are at present not available; so that we thought it best to avoid this difficulty by an assumption

\* See Section X, table (8).

† W. G. Shilling, *Phil. Mag.*, Vol. 3, 293, 1927

Table 9

Diameter of glass tubes	Frequency	V m/Sec	V <sub>s</sub> m/Sec	Tube-correction	Temp.
*1.04 cms.	988.5	339.4	342.4	3.00	18°C
*2.89 cms.	988.5	341.4	342.4	1.00	18°C
6.08 cms.	994.0	344.29	344.83	0.53	20°C

## XI

The Calculation of  $\Gamma$ 

In the calculation of  $\Gamma$ , the ratio of the two specific heats for saturated

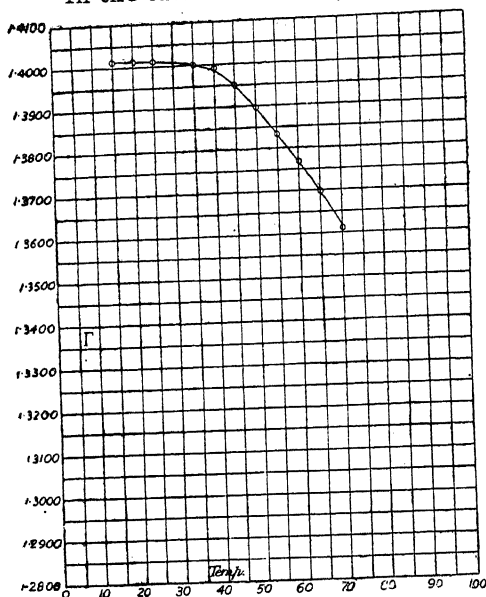


Fig. 5

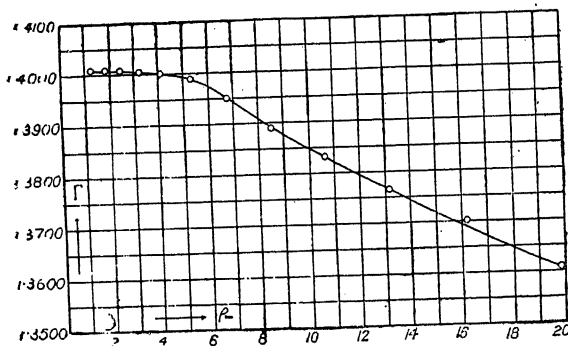


Fig. 6

The data for all such calculations and the results are given in table (10) below. Figs. (5) and (6) are the curves showing the variation of  $\Gamma$  with

air, the free-space velocities of sound at various temperatures were obtained from the smooth curve Fig. 3 drawn on a very large square paper. No correction for the imperfection of the saturated air deviating from the ideal gas law was made but simply the equation

$$V = \sqrt{\frac{P\Gamma}{\rho}}$$

was used.  $\rho_t$  the density of the saturated air at  $t^\circ\text{C}$ . was obtained by calculating  $\rho_0$ , the density of air at  $t^\circ\text{C}$ . and the partial pressure ( $P - p_{mt}$ ) and adding to this  $\rho_m$ , the density of the moisture present in the saturated air at  $t^\circ\text{C}$ . Here  $P$  is the total pressure of the mixture (i.e., saturated air) and  $p_{mt}$  is

the partial pressure of the moisture contained in the saturated air at  $t^\circ\text{C}$ . The densities of the moisture at various temperatures were calculated from Landolt's table, but more weight was given to the experimental values of the same obtained from a paper by J. H. Awbery (Proc. Phys. Soc., 44, 143, 1932).

\* Kaye and Sherratt, *Proc. Roy. Soc. A*, Vol. 141, p. 123, 1933.

Table 10

Temp.	$V_0$ m/sec in saturated air.	Total press. P mms.	Partial press. of moisture $p_m$ mms.	Partial press. of dry air $p_d$ mms.	Density of dry air at press. $p_d$ & $p_m$	Calculated density of moisture $p_m$	Experimental values of $p_m$	Density of saturated air $p = (p_m + p_d)$	$\gamma$ for saturated air.
15°C	341.55	742.1	12.78	729.3	$117.6 \times 10^{-5}$	$1.2 \times 10^{-5}$	...	$118.8 \times 10^{-5}$	1.4009
20°C	344.83	743.4	17.51	725.9	$115.0 \times 10^{-5}$	$1.8 \times 10^{-5}$	...	$116.8 \times 10^{-5}$	1.4008
25°C	348.15	744.3	23.69	720.6	$112.3 \times 10^{-5}$	$2.309 \times 10^{-5}$	$2.42 \times 10^{-5}$	$114.7 \times 10^{-5}$	1.4007
30°C	351.59	744.8	31.71	710.1	$108.9 \times 10^{-5}$	$3.041 \times 10^{-5}$	$3.17 \times 10^{-5}$	$112.1 \times 10^{-5}$	1.4005
35°C	355.30	743.3	42.02	701.3	$105.8 \times 10^{-5}$	$3.964 \times 10^{-5}$	$4.11 \times 10^{-5}$	$109.9 \times 10^{-5}$	1.4000
40°C	359.10	745.1	55.13	690.0	$102.4 \times 10^{-5}$	$5.118 \times 10^{-5}$	$5.28 \times 10^{-5}$	$107.7 \times 10^{-5}$	1.3989
45°C	363.05	745.6	71.6	674.0	$98.47 \times 10^{-5}$	$6.544 \times 10^{-5}$	$6.72 \times 10^{-5}$	$105.2 \times 10^{-5}$	1.3949
50°C	367.18	745.4	92.3	653.1	$93.93 \times 10^{-5}$	$8.299 \times 10^{-5}$	$8.47 \times 10^{-5}$	$102.4 \times 10^{-5}$	1.3893
55°C	371.70	745.0	117.8	627.2	$88.82 \times 10^{-5}$	$10.433 \times 10^{-5}$	$10.59 \times 10^{-5}$	$99.41 \times 10^{-5}$	1.3828
60°C	376.90	745.0	149.2	595.8	$83.12 \times 10^{-5}$	$13.011 \times 10^{-5}$	$13.13 \times 10^{-5}$	$96.25 \times 10^{-5}$	1.3705
65°C	382.80	745.0	187.3	557.8	$76.67 \times 10^{-5}$	$16.116 \times 10^{-5}$	$16.17 \times 10^{-5}$	$92.84 \times 10^{-5}$	1.3697
70°C	389.80	745.0	233.5	511.5	$69.27 \times 10^{-5}$	...	$19.76 \times 10^{-5}$	$89.02 \times 10^{-5}$	1.3618
75°C	398.60	745.0	299.1	445.9	$59.51 \times 10^{-5}$	...	$23.99 \times 10^{-5}$	$83.49 \times 10^{-5}$	
80°C	408.00	745.0	355.1	389.9	$51.28 \times 10^{-5}$	...	$28.92 \times 10^{-5}$	$79.7 \times 10^{-5}$	



the temperature and with the moisture content of the saturated air at that temperature respectively.

## XII

The Calculations of  $\gamma_m$ 

In order to calculate the values of  $\gamma_m$  for the moisture contained in the saturated air at different temperatures the values of  $\gamma_D$  for dry air at these temperatures were obtained by taking the most probable experimental values of the velocity of sound in dry air at the same temperature. But the selection of these values was restricted to only such tube experiments in which due care was taken to make the apparatus really air-tight. The values of  $\gamma_D$  for dry air were then calculated without applying any correction for the little deviation of dry air from the ideal gas law. The values of the velocity of sound in dry air taken from other workers together with the values of  $\gamma_D$  for dry air determined by other experimental methods are also set down in table (11) below.

Table 11

Temperature.	$V_0$ m/sec.	$\rho_D$ at 760 mms. pressure	$\gamma_D$ calculated.	Reference.
0°C	331.5	$129.3 \times 10^{-5}$	1.4023	Tube method, Kaye <sup>2</sup> and Sherratt.
14°C	339.8	$123.0 \times 10^{-5}$	1.4016	Tube method.
18°C	342.3	$121.4 \times 10^{-5}$	1.4027	Kaye <sup>2</sup> and Sherratt.
100°C	387.3	$94.64 \times 10^{-5}$	1.4017	" "
0°C	331.44	$129.28 \times 10^{-5}$	1.4017	In free air, Hebb.
Temperature.	$\gamma_D$ corrected.	..	$\gamma_D$ not corrected.	...
0°C	1.4025	..	1.4012	Lummer and Pringsheim.
0°C	1.4003	...	1.3992	Moody.
0°C	1.4034	...	1.4021	Partington.
0°C	1.4029	...	1.4016	Shields.
0°C	1.4026	..	1.4012	Hebb (1904)
0°C	1.4031	...	1.4017	Hebb (1919)

Having done this, we have calculated the values of  $\gamma_m$ , the ratio, of the two specific heats, for the moisture contained in saturated air at various temperatures by using Einstein's theoretical expression\*:

$$\frac{P}{\Gamma-1} = \frac{p_D}{\gamma_D-1} + \frac{p_m}{\gamma_m-1} \quad (5)$$

The data for all such calculations and the results are given in table (12) below:

Table 12

Temp °C.	Vap. pressure mms.	$p_m$	$\Gamma$	$\gamma_D$	$\gamma_m$
15°C	12.78	$1.2 \times 10^{-5}$	1.4009	1.4021	1.3421
20°C	17.51	$1.8 \times 10^{-5}$	1.4008	1.4020	1.3568
25°C	23.69	$2.42 \times 10^{-5}$	1.4007	1.4019	1.3672
30°C	31.71	$3.17 \times 10^{-5}$	1.4005	1.4018	1.3736
35°C	42.02	$4.11 \times 10^{-5}$	1.4000	1.4018	1.3723
40°C	55.13	$5.28 \times 10^{-5}$	1.3989	1.4017	1.3671
45°C	71.60	$6.72 \times 10^{-5}$	1.3949	1.4017	1.3406
50°C	92.30	$8.47 \times 10^{-5}$	1.3893	1.4016	1.3199
55°C	117.80	$10.59 \times 10^{-5}$	1.3828	1.4016	1.3054
60°C	149.2	$13.13 \times 10^{-5}$	1.3765	1.4015	1.3015
65°C	187.3	$16.17 \times 10^{-5}$	1.3697	1.4014	1.2993
70°C	233.5	$19.76 \times 10^{-5}$	1.3618	1.4013	1.2976
75°C	299.1	$23.99 \times 10^{-5}$	...	...	...
80°C	355.1	$28.92 \times 10^{-5}$	...	...	...
85°C	433.5	$34.67 \times 10^{-5}$	...	...	...
90°C	525.8	$41.28 \times 10^{-5}$	...	...	...

\* It may however be noted that the expression

$$\frac{1}{\Gamma-1} = \frac{\rho-\rho_D}{\rho_m-\rho_D} \cdot \frac{1}{\gamma_m-1} + \frac{\rho_m-\rho}{\rho_m-\rho_D} \cdot \frac{1}{\gamma_D-1}$$

due to Richarz (Ann. Phys. 1906) is the same as the expression (5).

Table 13

Temperature	$V_H$ , the free-space velocity in saturated air	$V_0$ , the free-space velocity in dry air	$V_H - V_0$ m/sec.
0°C	331.60	331.50	0.1
10°C	...	337.6	
15°C	341.55	340.40	1.15
20°C	344.8	343.5	1.32
25°C	348.1	346.35	1.75
30°C	351.5	349.3	2.2
35°C	355.3	352.1	3.2
40°C	359.1	354.95	4.15
45°C	363.05	357.8	5.3
50°C	367.2	360.7	6.5
55°C	371.7	363.4	8.3
60°C	376.9	366.2	10.7
65°C	382.8	368.9	13.9
70°C	389.8	371.6	18.2
75°C	398.6	374.3	24.3
80°C	408	377	about 31
(100°C)	(471.5)*	(387.3)†	(84.2)

## ACKNOWLEDGMENTS

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CHEMICAL EXAMINATION OF THE SEEDS OF  
*ABRUS PRECATORIUS*, LINN. PART III.  
THE CONSTITUTION OF ABRINE

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In part I of this series of investigations<sup>1</sup> the seeds of the important medicinal plant *Abrus precatorius*, Linn. of the natural order Leguminosae, or Jequirity as it is known in English and Rati in Hindustani, were chemically examined. A colourless crystalline nitrogenous compound in the form of slender needles was isolated and named as 'abrine' which is most probably the active principle of the seeds. The pharmacological examination of the substance is in progress in the King George's Medical College, Lucknow. In the present paper it is intended to elucidate the constitution of abrine as far as it has been possible by the preparation of some of its derivatives.

Abrine is present in the kernels of the seeds of *Abrus precatorius*. In pure form it has no smell and is tasteless. It crystallizes in minute silky white needles from alcohol. In water it is sparingly soluble in the cold and crystallizes well from a boiling solution. If it is allowed to crystallize very slowly extending over several days, abrine is obtained in the form of stout, long crystals in the form of stars. Careful crystallization may increase the length of the crystals from two to three centimetres. It has a high melting point at 295°. When heated over flame in a dry test tube abrine melts, becomes brown with decomposition and gives out white fumes which have a very disagreeable odour. The fumes condense in the cooler parts of the tube forming a yellowish white liquid. When heated with zinc dust it gives out the same smell as above along with that of ammonia.

Abrine forms mono-hydrochloric and mono-nitric acid salts with the corresponding acids. It thus behaves as a mono-basic compound. Abrine does not produce any coloration with neutral ferric chloride solution but forms a mono-acetyl and mono-phenylurethane, proving thereby the presence of an alcoholic hydroxy group. The formation of a dibromo derivative with bromine proves the presence of a double bond in abrine. This is also borne out by volumetric estimation of unsaturation in abrine. Abrine forms a mono-nitroso derivative, thus showing the presence of a

secondary amino group. The facts that abrine does not respond to the *ninhydrin-reaction* and its aqueous solution is not coagulated by tannic acid show that it has no amino-acidic group in it. Abrine forms a mono-nitro derivative and is readily oxidised by solution of potassium permanganate.

#### EXPERIMENTAL

It has already been shown<sup>1</sup> that the most suitable solvent for the extraction of abrine from the seeds of *Abrus precatorius* is alcohol, which takes out the substance in very small quantities. Solubility of abrine in water could not be used with advantage for its extraction as the powdered kernel of the seeds swells to a considerable extent in presence of water and working with such gelatinous mass becomes very difficult. The swelling is due to the presence of large quantities of albuminous and mucilagenous substances present in the kernels of the seeds.

For extraction of abrine, the hard yellow kernels were ground to a fine powder in a hand mill and was extracted in a big Soxhlet's extraction apparatus, with petroleum ether. When the oil was completely removed by the solvent the powder was freed from petroleum ether and exhaustively extracted with rectified spirit. With the progress of extractions the yellow colour of the powder became lighter and finally became white. The total alcoholic extract was concentrated to a small volume under reduced pressure. The thick brown syrup, which had a disagreeable odour, contained fine needle-shaped crystalline suspension. It was allowed to stand for about ten days when the quantity of the crystalline deposit increased. The product was separated and washed with benzene to remove oily contamination. It was next washed with small quantities of cold water which readily removed the sticky portions. The residue on crystallization from boiling water was obtained as fine silky white needles. As has previously been recorded, it has a molecular formula  $C_{12}H_{14}O_2N_2$  and has been named "abrine". The general reactions of abrine have also been recorded. Its solution is not coagulated by tannic acid solution and is non-respondent to the *ninhydrin-reaction* of amino-acids.

#### Abrine hydrochloride, $C_{12}H_{14}O_2N_2 \cdot HCl$

To 0.5 g. of abrine in a dry dish was added one c.c. of pure concentrated hydrochloric acid. Abrine immediately dissolved but very soon the whole of it was precipitated as white needle shaped crystals. The liquid contamination of the crystals was soaked out by means of filter papers. The product was dried in a vacuum desiccator over calcium oxide. It was then washed several times with dry ether to free it from traces of hydrochloric acid. It melted at  $221.5^\circ$  sharp. When slowly allowed to crystallize from fairly strong

hydrochloric acid, abrine hydrochloride separates in the form of long stout needles in starry clusters.

[Found : Cl=14.25 %;  $C_{12}H_{15}O_2N_2$  Cl, requires Cl=13.94%.]

Abrine hydrochloride dissolves in water but soon after a flocculent white precipitate separates. On analysis the precipitate has been found to be pure abrine and the filtrate contains hydrochloric acid. The combination of abrine with hydrochloric acid is therefore fairly weak and their detachment is effected in presence of water.

**Abrine nitrate,  $C_{12}H_{14}O_2N_2 \cdot HNO_3$**

0.5 g. of abrine was put in a small clean beaker and very dilute nitric acid was added slowly till the whole of abrine just dissolved. Excess of acid was avoided. The solution was kept for spontaneous evaporation. After few days long white needles of abrine nitrate separated. The crystals were dried within filter papers and finally in a vacuum desiccator over calcium oxide. It was finally washed with dry ether and on drying melted at  $143^\circ$  with decomposition. Abrine nitrate is very soluble in water and the combination of the salt is very stable in presence of the solvent.

[Found : N=15.42%;  $C_{12}H_{15}O_5N_3$ , requires N=14.94%.]

**Abrine picrate,  $C_{12}H_{14}O_2N_2 \cdot C_6H_3O_7N_3$**

1 g. of picric acid was dissolved in 30 c.c. of alcohol (98 %) and 0.5 g. of abrine was added. On slight warming, abrine dissolved and the colour of the solution slowly darkened and finally became orange-red. It was then heated to boil and allowed to stand overnight. Next morning orange-yellow crystalline plates in clusters were formed. The mother liquor was decanted off and the crystals were washed free from picric acid with dilute alcohol. The picrate weighed 0.9 g. and melted at  $194^\circ$  with decomposition. It was quite stable in presence of water in which it was very little soluble, forming faint yellow solution.

[Found : N=16.06 %; 15.92%;  $C_{18}H_{17}O_9N_5$ , requires N=15.66%].

The picrate was also prepared in glacial acetic acid from which two different types of crystals were obtained—(1) orange-red needles in form of stars, and (2) very closely packed soft yellow needles. Both melted at  $194^\circ$  with decomposition and the nitrogen content was 15.96 per cent and 15.89 per cent respectively. The orange-red variety changed colour and became perfectly yellow at  $120^\circ$ . Thus all the three varieties were mono-picric acid salts of abrine having only different crystalline modifications.

**Dibromo abrine,  $C_{12}H_{14}O_2N_2Br_2$**

0.5 g. of abrine was put in a dry flask and alcoholic solution of bromine was added in the cold. The colour of bromine was discharged and abrine

dissolved forming a light pink solution. Excess of bromine solution was added and was left for spontaneous evaporation at room temperature. After few days soft yellow plates settled at the bottom and the mother liquor remained brown. Addition of water did not separate more of the bromo derivative. Some acetone was next added when the solid deposit became colourless and the brown mother liquor was decanted off. It was thus freed from bromine by two more washings with acetone. The product on drying was obtained as whitish microcrystalline powder. It slowly started turning dark from  $220^{\circ}$  and melted between  $241-42^{\circ}$ , with decomposition.

[Found Br. = 42.7 %;  $C_{12}H_{14}O_2N_2Br_2$ , requires Br = 42.3%.]

The volumetric estimation of unsaturation in abrine was carried out as follows:—0.2974 g. of abrine was dissolved in 10 c.c. of carbon tetrachloride in a stoppered 250 c.c. measuring flask and N/3-bromine (20 c.c.) in the same solvent added and the mixture allowed to stand in a dark place for 24 hours. The mixture was then cooled in ice and water (25 c.c.) quickly added and well shaken and then 10 per cent potassium iodide solution (25 c.c.) with water (75 c.c.) introduced and the whole thoroughly agitated. The iodine thus liberated was titrated against N/10 sodium thiosulphate. After titration, 2 per cent potassium iodate (5 c.c.) was added and the titration repeated. Twice this value was deducted from the above titration value and the equivalents of bromine atoms taken up by abrine molecule calculated, which came to 1.97. This means one double bond in abrine.

#### Nitro-abrine, $C_{12}H_{13}O_2N_2.NO_2$

1 g. of abrine and 20 c.c. of nitric acid (d. 1.2) was put in a nitration flask. Abrine dissolved with evolution of heat and the colour of the solution became orange-red. In about an hour the whole of abrine was dissolved. It was refluxed for about 5 hours over water bath. On cooling a pasty brown mass settled at the bottom. The mother liquor on dilution with water deposited a flocculent yellow mass. The precipitate was washed, dried and crystallized from dilute alcohol. When heated, it shows no sign of melting, darkens at about  $185^{\circ}$  and finally decomposes at about  $220^{\circ}$ , being then a carbonaceous powder. The product was proved to be a nitro compound by silver deposit test (zinc, alcoholic silver nitrate and substance).

[Found N = 16.3 %;  $C_{12}H_{13}O_4N_3$ , requires N = 16.0 %.]

#### Nitroso-abrine, $C_{12}H_{13}O_2N_2.NO$

To a cooled solution of 0.5 g. of abrine in 20 c.c. of 10 per cent acetic acid was added a well cooled solution of 0.25 g. of sodium nitrite in 10 c.c. of water.

An yellow coloured precipitate was formed which was filtered. It was washed with 5 per cent acetic acid and then with water. It was dried in a vacuum desiccator when it melted at  $121^{\circ}$ .

[Found N=17.2 % ;  $C_{12} H_{13} O_3 N_3$ , requires N=17.0 %.]

**Acetyl abrine,  $C_{12} H_{13} ON_2 \cdot O \cdot COCH_3$**

1 g. of abrine was refluxed with 10 c.c. of acetic anhydride and fused sodium acetate for about an hour. It was cooled. On addition of cold water a brownish yellow paste separated, which solidified becoming brittle on long standing. It was twice crystallized from dilute alcohol and animal charcoal, when it was obtained as a white microcrystalline power. It melted at  $286-287^{\circ}$  with decomposition.

[Found N=10.9 % ;  $C_{14} H_{16} O_3 N_2$ , requires N=10.8 %]

**Abrine-phenylurethane,  $C_{12} H_{13} ON_2 \cdot O \cdot CO \cdot NHC_6H_5$**

0.7 g. of abrine and 10 c.c. of phenylisocyanate was kept in a dry flask and refluxed over water-bath, carefully avoiding the entry of water vapour into the flask. Abrine dissolved slowly and the colour of the mother liquor became yellow. On allowing the product to stand at ordinary temperature for some time the phenylurethane product crystallized out which was filtered off and washed with benzene till the smell of phenylisocyanate had completely disappeared. It was recrystallized from alcohol, when it was obtained as white needles. It melted at  $271^{\circ}$ .

[Found N=12.8 % ;  $C_{19} H_{19} O_3 N_3$ , requires N=12.5 %.]

The author wishes to express his indebtedness to the "Lady Tata Memorial Trust" of Bombay for a scholarship which enabled him to take part in the investigation.

*Reference*

1. Ghatak and Kaul, *J. Indian Chem. Soc.*, 9, 383, 1932.





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NEW BLOOD FLUKES OF THE FAMILY SPIRORCHIDAE STUNKARD FROM INDIAN FRESH-WATER TORTOISES WITH DISCUSSION ON THE SYNONYMY OF CERTAIN GENERA AND THE RELATIONSHIPS OF THE FAMILIES OF BLOOD FLUKES.—PART II.

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Received November 7, 1933.

INTRODUCTION

While we described two new species of the new genus *Coeuritrema* in a paper published in Vol. 2, No. 4 of this journal, we discussed the systematic position of that genus and relationships of the family Spirorchidae with the Schistosomatidae. It was shown in the course of discussion that the subfamily Hapalotreminae represents the primitive blood flukes, from which are evolved along one line the Schistosomidae and along the other close to the subfamily Spirorchinae, the blood flukes of fishes belonging to the families Aporocotylidae and Sanguinicolidae. In this paper are described four new species of blood flukes assigned to a new genus *Plasmiorchis*, which as will be seen from the description and subsequent discussion is closely related to the American genus *Spirorchis*. The resemblance between these two genera is so close that at first sight it appeared desirable to create two subgenera under the genus *Spirorchis*, one for the American species and the other for Indian forms described in this paper. But the closer examination revealed that the presence of a ventral sucker and forwardly directed loops of the intestinal caeca, one on each side of the oesophagus, are such constant features of the Indian species as to necessitate their inclusion in a separate genus, which we call *Plasmiorchis* on account of their habitat in the blood plasma of their hosts.

Odhner in 1911 while discussing the relationships of *Aporocotyle* with *Sanguinicola* pointed out that the H-shaped gut of the latter is derived by a great reduction in length of that of the former. He also mentioned that the

condition of the alimentary system in *Deontacylix* shows an intermediate condition between that of *Sanguinicola* and *Aporocotyle*. It seems obvious that we have got a descending series in the evolution of the alimentary system of the four genera of suckerless trematodes hitherto known, *Aporocotyle*—*Psettarium*—*Deontacylix*—*Sanguinicola*, all blood flukes of fishes. The presence of forwardly directed loops at the origin of caeca in *Plasmiorchis* gives us a clue to the origin of the anterior blindly ending lobes of the gut of the above-mentioned genera. It is, therefore, near the subfamily Spirorchinae particularly its genus *Plasmiorchis*, where we have to look for the ancestors of *Aporocotyle* and its descending series of genera *Psettarium*, *Deontacylix*, and *Sanguinicola*.

In view of the recent addition of a number of genera, the classification of the family Spirorchidae as given by Stunkard in 1923 has been revised. The genera *Henotosoma* Stunkard and *Haematotrema* Stunkard are held identical with the genus *Spirorchis* and are merged into it. The genus *Tremarhynchus* Thapar is held synonymous with *Coeuritrema*, the latter name being accepted on the basis of priority. *Tremarhynchus indicus* is consequently assigned as the third species to that genus under the name of *Coeuritrema indicus* (Thapar, 1933).

***Plasmiorchis orientalis* nov. gen., nov. spec.**

(Figs. 1, 4, 8, 9)

Three mature specimens were obtained in August 1931 and two in September 1932 from the ventricle of the heart of two water tortoises of the species *Kachuga dhongoka* at Allahabad. The blood flukes came out as soon as the ventricle was opened in salt solution and appeared inactive indicating no movements. The body is elongated fusiform or spindle-shaped, slightly tapering towards ends like that of the genus *Spirorchis*; it is thin specially at edges, flattened and transparent with the anterior end a little more pointed than the posterior. The size is very small, measuring 2.26\*—3 in length and 0.4–0.6 in greatest breadth, which lies about the middle of body length. The breadth measures 0.38–0.5 in the region of the intestinal bifurcation, 0.27–0.56 in that of the ventral sucker and 0.34–0.56 in that of the ovary, varying within the narrow limits of 0.33–0.4 or 0.5–0.6 between the intestinal bifurcation and the ovary. The body wall is covered with very thin cuticle and is devoid of papillae, but it is covered with fine needle-like spines, which hardly project outside it. The musculature of the body is poorly developed.

The oral sucker is oval, longer than broad and protrusible, measuring 0.102–0.108 in length and 0.066 in maximum breadth. It protrudes ordinarily

\* All measurements are given in millimeters.

only a little in front of the anterior end; in none of my specimens it is half or entirely protruded. The ventral sucker is well developed, protrusible and rounded, measuring 0.128–0.144 in diameter: in one specimen of 2.5 length it measures 0.081 in length and 0.087 in breadth, while the oral sucker in this specimen measures 0.102 in length and 0.066 in breadth. The ventral sucker lies 0.192–0.272 behind the intestinal bifurcation and 0.73–0.75 behind the anterior end, *i.e.*, at about one third body length from the anterior end: in one specimen, however, it lies between one third and one fourth body length from the latter. The pharynx is absent. The oesophagus, 0.4 in length and 0.066–0.069 in greatest breadth is long and sinuous with two to four bends, extending up to 0.48 length of the body from the anterior end, *i.e.*, about anterior one sixth body length. It is surrounded by deeply staining salivary gland cells, which are found in large numbers around its posterior part. Its inner wall appears plicated on account of the continuous discharge of salivary secretion through it into the lumen: the plications are more pronounced in the terminal part immediately in front of the intestinal bifurcation. At the point where it bifurcates into the caeca, it gives off behind the origin of the latter a small median pocket, the oesophageal vesicle. The caeca do not pass laterally as soon as they arise as in the genus *Spirorchis*, but they turn abruptly forwards running one on each side of the oesophagus for nearly one third or one half of its length and then bend downwards forming characteristic U-shaped loops as they continue their course to the hinder end. The presence of these forwardly directed loops is a characteristic feature of all the species belonging to the new genus. The caeca then run almost straight but for a short outward bend displayed by the left caecum in the region of the genital pore, the genital loop and terminate near the hinder end, where they converge a little towards each other lying parallel for a short distance. They are simple without diverticula, having more or less uniform breadth of 0.075, and lie half way between the body wall and median line except near their ends.

The excretory pore is slightly dorsal at the posterior end. The excretory bladder has a very small stem bifurcating into two wider branches, which could be traced up to the blind ends of the caeca. The glandular vesicle, which Stunkard considers as the lymph vesicle in the genus *Spirorchis* is not seen in sexually mature specimens. In immature specimens, however, it is present at the hinder end in the median line behind the vitelline reservoir, having a curved S-shaped appearance.

Main parts of the nervous system are visible in entire mounts. The oesophageal commissure is fairly prominent. It lies 0.096 distance behind the oral sucker, *i.e.*, at about one fourth distance from it and is slightly swollen on each side to form an indistinct ganglionic mass from which the main lateral nerve arises. The lateral nerves run posteriorly close outside the caeca on the ventral surface of the body as in *Spirorchis*.



The reproductive organs resemble essentially those of the genus *Spirorchis*. The testes, 5-7 in number, lie in a linear series in the median plane usually in close contact with one another, almost filling the intercaecal region between the ventral sucker and ovary. They are irregularly lobed, elliptical or ovoid in shape, flattened and broader than long, measuring 0.14-0.24 in length and 0.208-0.24 in breadth. The anterior and posterior testes are larger than the middle ones. The anterior testis lies a little behind the ventral sucker, 0.03-0.12 distance behind it and 0.033-0.53 behind the intestinal bifurcation. The testicular area occupies a little less than half the length of the body. There is no vas deferens. The vesicula seminalis, filled with sperms, is pear-shaped with the widest anterior end pressed against the left part of the posterior margin of the hindmost testis, situated usually to the left side and slightly overlapped by the inner margin of the ovary, filling almost the entire space between it and the left caecum. In a few specimens, however, it lies almost entirely ventral to the ovary, commencing a little in front of the latter, from the posterior margin of the hindmost testis. It extends backwards as far as the posterior limit of the ovary or a little behind it to the left side near the left caecum, where it enters the cirrus sac, measuring 0.27 in length and 0.075-0.105 in maximum breadth in its widest anterior end. The cirrus sac is extremely small with poorly developed musculature, somewhat oval or pear-shaped with the narrow end opening at the genital pore, and situated beneath or close inside the left caecum, measuring 0.09-0.13 in length and 0.03-0.054 in maximum breadth at its end near the vesicula seminalis. It contains inside a small vesicula seminalis interna of rounded or oval outline and of 0.045 diameter, followed by a sharply constricted ductus ejaculatorius. The latter is pear-shaped, measuring 0.09 in length and 0.03 in greatest breadth near its proximal end. The genital opening lies 0.28-0.29 distance in front of the hinder end, and a little, *i.e.*, 0.128 distance behind the ovary, to the left side beneath the left caecum, where the latter is slightly bent outwards to form the characteristic genital loop. The cirrus sac opens anteriorly to the metraterm at the genital pore. The prostate gland cells are absent.

The ovary is much lobed, and lies median or slightly to the right side with its outer wall close inside the right caecum, immediately behind the hindmost testis at a distance of 0.4-0.6 from the posterior end, *i.e.*, at about one fifth body length from it. It overlaps partly or entirely the basal part of the vesicula seminalis, measuring 0.24 by 0.176, 0.176 by 0.112 and 0.9 by 0.9 in size in the three specimens examined for the purpose. The oviduct originates from the middle of its posterior margin, and after running a short distance dorsally backwards joins the receptaculum seminis which is filled with sperms. The receptaculum seminis is of an oval or spherical shape, and lies to the right side close behind the ovary, measuring 0.045-0.06 in

length and 0.05—0.075 in maximum breadth. We follow Ejsmont in calling this vesicle, the receptaculum seminis instead of receptaculum seminis uterinum as Ward and Stunkard have called it. The oviduct leaves the latter at the hinder end, and receives the small Laurer's canal; it then continues its course slightly to the left side, a little behind the level of the genital opening to receive the yolk reservoir, where it sharply turns forwards to open into the broad oval ootype or uterus, which contains a single large ovum. The uterus lies transversely, closely in front of the union of the transverse vitelline ducts, behind the receptaculum seminis, and enters terminally into a small metraterm with thin muscular walls. The latter opens to the exterior at the genital opening. The ovum is large, non-operculated and oval without filaments or spines, measuring 0.102—0.12 in length and 0.042—0.057 in maximum breadth.

The vitellaria are voluminous, and occupy lateral areas surrounding the caeca, extending from the middle of the oesophagus; *i.e.*, from the forward limits of the anterior loops of the caeca to almost the posterior end of the body. The follicles of small size lie mainly outside the caeca forming a continuous linear mass, the extracaecal areas, but they enter also the intracaecal region both dorsally and ventrally to form intracaecal areas. At the posterior end they generally meet in the intracaecal region between the blind ends of the caeca. The transverse vitelline ducts arise slantingly at about the level of the genital opening, the right a little in front of the left one, and unite closely behind the uterus near the ventral wall to form the backwardly directed vitelline reservoir, the narrow anterior end of which bends forwards on the dorsal side to open into the oviduct, near its junction with the uterus.

Four immature specimens were obtained from the ventricle of the heart of two *Kachuga dhongoka* (2 from each) dissected in November 1931. The body measures 1.5—1.9 in length and 0.37—0.54 in maximum breadth, which lies in the region between the ventral sucker and the ovary. The shape is elongated and fusiform with bluntly pointed ends. The oral sucker is oval and partly protrusible, measuring 0.09 by 0.05, and 0.1 by 0.06 in size in the two specimens examined for the purpose. The ventral sucker, 0.08—0.09 in diameter lies at about one third body length from the anterior end and 0.16—0.17 behind the intestinal bifurcation. The oesophagus, oesophageal vesicle and intestinal caeca with their characteristic loops are similar to those in mature specimens. The testes, 5—7 in number, are fairly large in size, containing mature sperms. The anterior testis, which is the smallest lies 0.096—0.135 behind the ventral sucker. The vesicula seminalis and ovary though small are developed in only one of the four specimens. The cirrus sac is indistinguishable. The vitellaria are entirely absent. The receptaculum seminis, present in only one specimen which possesses a very small slightly lobed ovary, is filled with sperms. From the study of immature specimens it is clear that the male reproductive organs are developed first and that the sperms for fertilisation are received in the

receptaculum seminis before the ovary is fully developed and functional. The vitellaria are developed last

Host—*Kachuga dhongoka*

Habitat—Ventricle of heart.

Locality—Allahabad, India.

***Plasmiorchis pellucidus* sp. n.**

(Figs. 2, 5, 10)

Two mature and one immature specimens of this species were obtained from the ventricle of the heart of *Kachuga dhongoka* in November 1931. The body is very thin and transparent, measuring 3.3.26 in length and 0.64-0.68 in greatest breadth, which lies in the region from the ventral sucker to the ovary. The shape is fusiform with bluntly pointed ends as in the genus *Spirorchis*.

The oral sucker is oval, longer than broad, and protrusible, measuring 0.126-0.132 in length and 0.087-0.09 in greatest breadth. The ventral sucker is absent in mature specimens, but its position is indicated by a definite clear region devoid of musculature or body parenchyma. This region where the ventral sucker was present and has obviously dropped off leaving a clear empty space in the body, we prefer to call the ventral sucker area. In immature specimen, however, the ventral sucker is present. This condition shows clearly how the ventral sucker has been secondarily lost in the genus *Spirorchis*. The ventral sucker area lies at about the end of the anterior one third body length, and measures 0.24-0.32 in diameter. The pharynx is absent. The oesophagus is long and sinuous with four bends, measuring 0.64 in length. It is more or less of uniform breadth except anteriorly where it is slightly narrower. It is surrounded by salivary gland cells, which are more numerous and densely crowded around its posterior end, before it passes into the vesicle; its inner wall appears plicated on account of the salivary secretion, which passes through it into the lumen. The oesophageal vesicle is well developed, measuring 0.096 in length and 0.08 in breadth. The intestinal caeca arise at the junction of the vesicle with the oesophagus and soon turn abruptly forwards running parallel to the latter for about posterior half of its length till they bend downwards forming the characteristic loops on their way backwards, ending almost at the hinder end of the body. The caeca are simple without diverticula, much narrower than the oesophagus, and run almost straight till the genital pore where the left caecum is slightly bent outwards to form the inconspicuous genital loop, which is less marked than that in the previous species. They lie almost half way between the body wall and the median line, converging slightly inwards towards each other behind the genital opening. The excretory pore is slightly dorsal. The glandular vesicle

is not visible in mature specimens. The nervous system is similar to that of the previous species

The testes, 8—9 in number, lie in a linear series in the median line, close behind and in contact with one another, separated on either side by a considerable distance from the corresponding intestinal caecum. They are of varying shapes, but usually ovoid with entire margins, and broader than long, measuring 0·09—0·165 in length and 0·105—0·175 in maximum breadth; the third and fourth testes are usually the smallest. The first testis lies a little behind the ventral sucker area, 0·08 distance behind it; the testicular area occupies a little less than half the length of the body. The vas deferens is absent. The vesicula seminalis is filled with sperms, and lies as in the previous species immediately behind the hindmost testis with its anterior dilated part interposed between it and the ovary in the median line. It is pear-shaped with the narrow tubular part directed to the left side towards the genital opening, where it enters the small cirrus sac; anteriorly it is overlapped for the greater part of its length by the left half of the dorsally situated ovary. The cirrus sac, 0·18 in length and 0·045 in maximum breadth, is tubular, commencing a little behind the ovary, at about the level of the receptaculum seminis near the left caecum. It contains inside a small portion of the vesicula seminalis, constricted off from the larger distally situated ductus ejaculatorius filled with sperms. The genital opening lies to the left side, a little behind the receptaculum seminis, at a distance of 0·35 from the hinder end beneath the left caecum, where the latter slightly bends outwards to form the inconspicuous genital loop.

The ovary lies median or slightly to the right side with its outer wall close inside the right caecum, closely behind the hindmost testis, at a distance of 0·5—0·58 from the hinder end, *i.e.*, about one fifth body length from it. It is divided into four or five lobes, and has nearly equal long and broad diameters, measuring 0·17—0·2 by 0·17—0·2 in size. The oviduct originates from the middle of the hinder margin of the ovary, and runs for a short distance before it joins the oval or spherical receptaculum seminis filled with sperms. The receptaculum seminis, 0·072—0·8×0·09 in size, lies closely behind the ovary, to the right side near the right caecum, at 0·43 distance in front of the hinder end. The oviduct leaves the receptaculum seminis at the hinder end near its outer margin, and passes a little backwards to the median line behind the level of the genital opening to join the narrow anterior end of the vitelline reservoir; soon after it sharply turns on itself to continue forwards as the prominent uterus, distended with the single ovum contained in it. The uterus lies transversely as in the previous species, a little behind the receptaculum seminis to the left side, and terminates into the short narrow metraterm, which opens to the exterior at the genital opening. Laurer's canal is very small and inconspicuous. The shell gland cells are absent. The

ovum is large, non-operculated and oval without filaments, measuring 0.136 in length and 0.095 in maximum breadth.

The vitellaria are well developed, extending from about the middle of the oesophagus, *i.e.*, from the anterior limits of the anterior loops of the caeca to a little in front of the hinder end of the body. They are composed of follicles of fairly large size, restricted mostly to the extracaecal region and overlapping the caeca at places. The follicles hardly extend inside the caeca, except behind the vitelline reservoir and in the region of the anterior loops, where they tend to meet in the median line. The transverse vitelline ducts arise obliquely, the right one at about the level of the genital opening and the left one a little behind it, and unite just behind the uterus near the ventral body wall to form the vitelline reservoir, which ends blindly to the left side near the left caecum.

*Plasmiorchis pellucidus* is distinguished from *P. orientalis* in a number of important features. The ventral sucker is absent in mature specimens; its position, however, can be easily determined by the presence of a clear transparent region free from the parenchyma, which is called the ventral sucker area. The testes are larger in number and are entire or slightly lobed, whereas in *P. orientalis* they are much lobed and lie quite apart from one another, filling almost the entire breadth of the intracaecal region. The length of the oesophagus and the anterior loops of the caeca is greater in *P. pellucidus* than in the other species. The vitelline follicles are mostly restricted to the extracaecal areas and do not enter the intracaecal region outside the testes as in *P. orientalis*.

Host—*Kachuga dhongoka*.

Habitat—Ventricle of heart.

Locality—Allahabad, India

### ***Plasmiorchis hardellii* sp. n.**

(Figs 3, 6, 11, 12, 13)

These blood flukes were collected in November and December 1931 and August 1932 from the ventricle of the heart and aortic arches of the water tortoises *Hardella thurgii*, which are not commonly available at Allahabad. Out of the five tortoises examined three were found infected, with nine, one and five parasites each; of these we possess nine specimens in entire mounts and others in longitudinal and transverse sections. When freed in normal salt solution they move slowly with gliding movements. The body is thin, transparent, elongated and elliptical in shape with rounded anterior and posterior ends; the anterior end is generally broader and more rounded, but sometimes it is bluntly pointed on account of the partly protruding oral sucker. The size is larger than that of the other species, measuring in entire

TABLE SHOWING MEASUREMENTS OF *Plasmodiopsis hardellii*.

No.	Length of body.	Breadth in the region of ventral sucker.	Greatest breadth.	Oral sucker.	Ventral sucker.	Length in front of ventral sucker.	Length behind ventral sucker.	Genital opening: distance in front of hinder end.	Ovary: distance in front of hinder end
1.	5.28	1.36	1.57	0.24 × 0.176	0.41 × 0.64	1.44	3.42	0.62	0.75
2.	5.15	1.23	1.39	0.22 × 0.176	0.38 × 0.57	1.44	3.33	0.6	0.72
3.	4.38	1.02	1.34	0.16 × 0.128	0.304 × 0.368	1.23	2.816	0.624	0.672
4.	4.53	1.104	1.06	0.24 × 0.192	0.37 × 0.48	1.12	3.04	0.45	0.528
5.	3.85	1.088	1.18	0.21 × 0.176	0.368 × 0.48	1.12	2.37	0.38	0.45

mounts 5.55—5.28 in length and 1.18—1.57 in maximum breadth, which lies in the region midway between the ventral sucker and the ovary, *i.e.*, about the middle of the body length. The body wall has a thin muscular layer, which is covered outside by a thin cuticle. It is devoid of papillae, but it is armed with fine needle-like spines, which hardly project outside it.

The oral sucker is longer than broad and slightly protrusible, measuring ordinarily 0.24 in length and 0.176 in maximum breadth; in one specimen it measured 0.16 by 0.13 and in another 0.21 by 0.19 in size. The ventral sucker is broader than long and about double the size of the oral sucker, measuring 0.3—0.4 in length and 0.36—0.64 in maximum breadth. It is muscular, having a well developed layer of radial muscles with an outer thin layer of longitudinal muscle fibres, and lies 0.29 distance behind the intestinal bifurcation, at about one third body length from the anterior end. Its exact position can be seen from the table in which the length of the body in front and behind it is given in specimens of different lengths. The pharynx is absent. The oesophagus is long and slightly undulating with two or three bends, measuring 0.67—0.96 in length, *i.e.*, one fifth to one sixth part of the body length. It gradually increases in breadth towards the hinder end, and is surrounded by deeply staining salivary gland cells, which are found in much larger numbers around its hinder part. Its inner wall is plicated as in the other species. The oesophageal vesicle is well developed. The intestinal caeca arise at the junction of the vesicle with the oesophagus, and soon turn forwards to form the characteristic loops as described in the other species, which run parallel to the oesophagus for about the posterior three fourth of its length. The caeca of a much narrower calibre than the oesophagus possess small irregular diverticula, and form well defined loops in the region of the genital opening, close behind the ovary, before they terminate near the hinder end of the body. The genital loop formed by the left caecum has a characteristic semicircular shape, and is much wider than the corresponding loop of the right caecum, which lies closely behind the ovary with the bend directed inwards. These loops are specially well developed in this species; their hinder border marks out the anterior limit of the region, in which the glandular sac lies.

The excretory opening lies dorsal a little in front of the hinder end. The excretory bladder has a very short median stem, which bifurcates into two long narrow ducts running one on each side outside the caeca throughout the body length. The glandular vesicle is a large convoluted tubular mass, occupying the entire intracaecal space behind the genital loops of the caeca, *i.e.*, behind the ovary and the genital opening, and measuring 0.54 in length and 0.51 in maximum breadth near its anterior end. The presence of a large convoluted glandular vesicle, which is closely pressed against the walls of the caeca near their blind ends is a distinctive feature of this species. Histologically the walls of this tubular mass consist of an epithelium of cells, which

have lost their outlines and in most cases their nuclei; a few nuclei are, however, present in a degenerate condition here and there in the epithelium, which is apparently converted into a secretion. The secretion also fills the lumen of the vesicle in adult specimens. There is no muscular layer outside the epithelium. The glandular tubular mass ends blindly in the body, and is not of the nature of a lymph receptacle. It appears to be an important gland, which secretes a fluid of a colloidal nature, to be possibly absorbed within the body for some unknown physiological needs of the animal.

The nervous system resembles closely that of the other species. The oesophageal commissure, conspicuous in entire mounts, lies close in front of the anterior limits of the anterior loops of the intestinal caeca, 0.021 distance behind the anterior end of the oesophagus and 0.41 behind the anterior end of the body.

The testes, 19–21 in number, lie in a linear series in the median line, 0.064–0.096 distance behind one another, separated on either side by a moderate distance from the corresponding intestinal caecum. They are much broader than long with a characteristic band-like irregular form, narrow antero-posteriorly, thicker in the middle, pointed or somewhat notched at their lateral ends, and produced into one or two very short-pointed outgrowths near the middle region. Four or five anterior and a few posterior testes have the smallest breadth, measuring 0.032–0.1 in length and 0.24–0.27 in greatest breadth. The largest testes, situated about the middle of the row measure 0.032 in length and 0.4–0.48 in maximum breadth. The foremost testis lies a little distance behind the ventral sucker (0.128 behind it) and a little behind one third body length from the anterior end; the hindmost testis lies a little in front of the ovary and at a distance of 0.88 from the posterior end of the body. The testicular area occupies a little less than half the length of the body. In immature specimens the testes are seen developing in irregular chink-like spaces, marked out in the central deeply staining body parenchyma behind the ventral sucker. The vas deferens is absent and the vesicula seminalis poorly developed. In none of my specimens the latter is visible on account of the absence of sperms in it; it is represented, however, by a small median space between the hindmost testis and the cirrus sac. The cirrus sac is large, and unlike that of the other species has thick muscular walls composed of an inner layer of longitudinal and outer layer of circular muscle fibres. It is situated obliquely with the basal end near the median line just in front of or in level with the anterior margin of the ovary, a little behind the hindmost testis, and the terminal end to the left side close inside the left caecum within the region enclosed by its genital loop, 0.16 distance from the left body margin, and 0.5–0.67 distance in front of the hinder end.

The ovary lies to the right side closely inside the right caecum, 0.112 behind the hindmost testis and one fifth to one seventh part of the body



length in front of the hinder end, 0.45–0.75 in front of the latter. It is irregularly lobed and small in size, measuring 0.075–0.08 in length and 0.09–0.2 in maximum breadth. The oviduct arises from its inner margin, and runs backwards for a short distance to become enlarged into the receptaculum seminis, which in most of the specimens is not filled with sperms. The receptaculum seminis is somewhat pear-shaped, measuring 0.072–0.075 in length and 0.03–0.033 in maximum breadth. It becomes narrowed at its hinder end to pass into the uterus, which runs transversely from the median line to the left side. The uterus, 0.108 in length and 0.021–0.024 in breadth, is thin walled lined with parenchymatous cells, and passes at its terminal end into the metraterm of 0.108 length, which is lined internally with a thin cuticular layer, and shows peculiar elevations and depressions of its modified epithelium devoid of nuclei. The metraterm, however, is devoid of a muscular layer except in the small terminal part, the muscular tube, which is covered by a strongly developed musculature, functioning as a sphincter; the latter has no epithelial layer inside, and runs vertically downwards to open at the genital opening. The shell gland cells are absent. A small inconspicuous Laurer's canal is present. The ovum is large, elongated oval in shape, and non-operculated without filaments, measuring 0.081 in length and 0.03 in greatest breadth. Only one ovum is contained at a time in the uterus or the metraterm.

The vitellaria are well developed in a few specimens in my collection. They are situated laterally as in the other species, commencing a little in front of the middle of the oesophagus and terminating at the blind ends of the caeca. The follicles are smaller than those of the other species and are mostly aggregated outside the caeca, extending slightly inwards in the lateral areas outside the testicular zone. The transverse vitelline ducts lie just in front of the convoluted glandular vesicle, the right immediately behind the ovary and the left a little further behind. The vitelline reservoir lies immediately behind the transverse ducts in the median line, ending blindly just in front of the glandular vesicle. A fairly large number of specimens possessed the testes, cirrus sac, ovary, uterus and metraterm, but they lacked the vitellaria, which were obviously not developed. In a few specimens the latter were found developing as a mass of nuclei situated here and there in the extracaecal and intracaecal areas. The vesicula seminalis and receptaculum seminis were free from sperms in these relatively immature worms.

*Plasmiorchis hardellii* is distinguished from the other species in the large size and shape of the body, characteristic irregular shape of the testes, enormous development of the convoluted glandular vesicle, large size of the cirrus sac, small size of the ovary, slight development of the vesicula seminalis and presence of large well marked genital loops of the caeca in the region of the genital opening. The cirrus sac has thick muscular walls, while its musculature in the other species is weakly developed as in the genus

*Spirorchis*. The vitellaria are composed of follicles of small size. The metraterm is characterised by the presence of a highly modified epithelium showing elevations and depressions. It is devoid of musculature except in the terminal part, which has a strongly developed musculature functioning as a sphincter. The intestinal caeca have indented margins or small diverticula throughout their course.

Habitat—ventricle of heart and aortic arches.

Host—*Hardella thurgi*.

Locality—Allahabad, India.

***Plasmiorchis obscurum* sp. n.**

(Fig. 7)

Three immature specimens of this species were obtained from the ventricle of the heart of two specimens of *Kachuga dhongka*, one from one host and two from the other. Body length 3–3.2, maximum breadth 0.64 about the middle of body length; breadth in the region of ventral sucker 0.5. Body thin, transparent, elongated and spindle-shaped with bluntly pointed ends, broader near the anterior than near the posterior end. Suckers well developed. Oral sucker oval, entirely protrusible and twice as long as broad, measuring 0.176 × 0.08. Ventral sucker a little in front of one third body length, circular in outline, 0.144 in diameter. Pharynx absent. Oesophagus long, slightly undulating, nearly straight in the extended condition, 0.56 in length and 0.128 in maximum breadth, surrounded by salivary gland cells. Oesophageal vesicle well developed, 0.096 in length and 0.128 in breadth, ending 0.144 distance in front of ventral sucker. Anterior loops of intestinal caeca one on each side of oesophagus as in the other species, 0.384 in length. Intestinal caeca approaching towards each other and slightly undulating near their hinder end, with small outgrowths given off mainly from the inner walls and ending a little in front of the hinder end. A small, tubular, nearly straight glandular vesicle present in the median line at the hinder end of the body. Excretory bladder with a very short median stem bifurcating just behind the glandular vesicle into two long narrow ducts extending throughout the body length outside the intestinal caeca.

Intracaecal region between oesophageal pouch and rudiment of ovary a deeply staining mass of cells with rudiments of testes lying in a series in the median line. Testes rudimentary and hence small in size, about nineteen in number, separated from one another by 0.048–0.08 distance; posterior testes situated nearer one another than anterior testis. Ovary small and rudimentary. Vitellaria not yet developed.

The specimens though sexually immature with poorly developed genital organs, show certain well marked features, in which they are distinguishable

from the other species, such as the shape of the body, intestinal caeca undulating near the hinder end and provided with small outgrowths and large number of testes. This species differs from *P. hardellii* in size, shape of body, presence of a small glandular vesicle and in the size of the suckers and oesophagus. It comes nearer *P. orientalis* in shape and size of the body, presence of two suckers, size of ventral sucker, length of oesophagus and small size of glandular vesicle, but it differs in the intestinal caeca, which are undulating near the hinder end and possess small outgrowths. Moreover, it has a much larger number of testes than *P. orientalis*.

Habitat—Ventricle of heart.

Host—*Kachuga dhongoka*.

Locality—Allahabad, India.

### Diagnosis of the Genus **Plasmiorchis** N. G.

Spirorchinae: Hermaphrodite distome blood flukes; delicate musculature. Body thin, elongated, flattened, narrow and elliptical or spindle shaped as in *Spirorchis*; body wall covered with very thin cuticle having fine needle like spines hardly projecting outside it. Oral sucker oval and protrusible; ventral sucker present (absent only in adult *Plasmiorchis pellucidus*, though ventral sucker area present), situated at about one third body length from anterior end. Pharynx absent; oesophagus long, about one fifth to one sixth length of body, sinuous with two to four bends and surrounded by salivary gland cells, which are numerous near its hinder end; inner wall of oesophagus plicated; small median pocket the oesophageal vesicle present at the point of intestinal bifurcation a little in front of ventral sucker. Intestinal caeca do not pass laterally as they arise, but turn abruptly forwards for one third to three fourths length of oesophagus and then bend backwards to form forwardly directed U-shaped loops, one on each side, continuing their course backwards almost to posterior end of body and forming small or well marked loops, specially the left caecum, in the region of genital pore. Genital opening ventral to the left side, beneath or close inside left intestinal caecum, behind ovary and a little in front of hinder end. Testes in a large number, arranged in a linear series in the median plane in the intracaecal area, behind ventral sucker and in front of ovary, usually irregularly lobed and of varying shapes; testicular area occupies a little less than half the length of body; vas deferens absent; vesicula seminalis usually well developed, filled with sperms and pear-shaped with its widest anterior end in contact with the hindmost testis. Cirrus sac extremely small with weak musculature except in *P. hardellii*, in which it is fairly large with stout musculature (vesicula seminalis small in this species); vesicula seminalis interna and ductus ejaculatorious present. Ovary lobed, median or slightly to the right, immediately behind hindmost testis,

about one fifth body length in front of hinder end; receptaculum seminis oval or rounded immediately behind ovary; uterus very short distinguished by the presence of a single large ovum, just behind receptaculum seminis; metraterm very short. Laurer's canal present and shell gland cells absent. Transverse vitelline ducts slantingly situated, right a little in front of the left one, immediately behind uterus; yolk reservoir behind transverse ducts, ending blindly to the left side. Vitellaria voluminous and situated laterally, extending from about middle of oesophagus, *i.e.*, from anterior limits of forwardly directed loops of caeca to posterior end of body, outside or surrounding intestinal caeca. Excretory opening slightly dorsal at posterior end; excretory bladder with a very small median stem bifurcating into two long narrow ducts, one on each side of body outside caeca. Glandular vesicle in the form of a tubular mass usually present near hinder end behind ovary and genital pore.

Habitat—Ventricle of heart and main arteries.

Host—Water tortoises, *Kachuga dhongoka* and *Hardella thurgi*.

Locality—Allahabad, India.

*Type species*—*Plasmiorchis orientalis* sp. n.

#### Remarks on the Systematic Position of the Genus *Plasmiorchis* N. G., the Synonymy of Certain Genera and Classification of the Family *Spirorchidae*.

The genus *Plasmiorchis* belongs to the family Spirorchidae and the subfamily Spirorchinae, bearing a close relationship to the genus *Spirorchis* MacCallum 1918. It resembles the latter genus in the position and large number of testes, which lie in a linear series in the median plane, in the intracaecal area in front of the ovary and the genital pore. The vas deferens is absent, vesicula seminalis well developed and the cirrus sac small with poorly developed musculature as in *Spirorchis*. In *P. hardellii*, however, the vesicula seminalis is small and the cirrus sac relatively larger with strongly developed musculature. The ovary is always lobed and occupies the same position in the two genera, *i.e.*, median or slightly to the right, a little behind the hindmost testis. The size and position of the receptaculum seminis, uterus, vitelline ducts and vitelline reservoir are similar. The ovum is discharged singly and is without filaments. The vitellaria are voluminous and lateral; there is a close resemblance in the excretory system of the two genera. The glandular vesicle is also present near the hinder end of the body in both. The important features in which *Plasmiorchis* differs from *Spirorchis* are the presence of a ventral sucker (ventral sucker though absent in adult *P. pellucidus* is represented by a definite zone, the ventral sucker area) and forwardly directed loops at the origin of the intestinal caeca, one on each side of the oesophagus. It also differs in certain minor characters such as the

more forward extension of the vitellaria, which commence from about the middle of the oesophagus and the position of the genital opening, which lies a little behind the ovary and not in level with its caudal margin as in *Spirorchis*. The left intestinal caecum in *Plasmiorchis* gives off a loop, the genital loop in the region of the genital opening enclosing a space in which the cirrus sac and metraterm lie.

The genera *Henotosoma* Stunkard, 1922 and *Haematotrema* Stunkard, 1923 should be considered synonymous with the genus *Spirorchis*, which they resemble in the entire anatomy and topography of organs. The testes in these genera are large in number, and arranged in a linear series as in *Spirorchis*. The position of the ovary and the genital opening is also similar. The intestinal caeca arise just before the posterior end of the oesophagus and pass lateral about one half of the distance to the body wall, before they turn backwards, extending almost to the posterior end as in *Spirorchis*. They are also monostomes, lacking the ventral sucker. The only character in which they differ from the latter genus is the position of the testes in the posterior half of the worm. There are some minor points of difference between *Henotosoma* and *Haematotrema* in the number of testes and position of the genital pore; in the former the testes are ten in number, and in the latter four, situated in the anterior part of the posterior half of the body. The genital pore in *Haematotrema* lies a little in front of that in *Henotosoma*. In our opinion these differences are not important enough so as to be considered of generic rank, the genera *Henotosoma* and *Haematotrema*, therefore, are merged in the genus *Spirorchis*, the former reduced to *Spirorchis haematobium* (Stunkard, 1922) and the latter to *Spirorchis parvum* (Stunkard, 1923).

*Tremarhynchus indicus* Thapar, the account of which is published in June 1933 number of Helminthology, agrees closely in many features with the species of *Coeuritrema* described by me in May 1933. Both these genera possess two testes with the ovary between them, a large cirrus sac with the vesicula seminalis outside it, in front of the anterior testis and an eversible cirrus. The position of the genital pore in both the genera is sinistral and dorsal, close behind the ventral sucker near the middle of the body length. The important points of difference are the absence of receptaculum seminis, presence of shell glands and larger size of vitellaria in *Tremarhynchus*. Thapar makes no mention of the presence of salivary gland cells and metraterm in his species. The salivary gland cells are present without exception in all the families of blood flukes and it is impossible to believe that they are absent in *Tremarhynchus*. It appears likely that the vitellaria in the latter do not extend in front of the intestinal bifurcation and the salivary gland cells surrounding the oesophagus have been taken for the vitelline gland cells in that region. *Tremarhynchus indicus* resembles *Coeuritrema lyssimus* and *Coeuritrema odhnerensis* so closely that it is unlikely that the receptaculum

seminis, which is a characteristic feature of the latter two species, is absent in it. We also feel doubtful for the same reason about the existence of the shell gland cells and the absence of the metraterm in this species. Thapar's observation that in his species the two caeca run backwards to the posterior end as slender straight tubes except a little distance in front of the hinder end needs confirmation, because obviously the dorsal shifting of the genital pore in *Hapalorhynchus* and *Coeuritrema* has resulted in the formation of the characteristic loop of the left caecum towards the median line in the region of the pore. Apart from these differences *Tremarhynchus indicus* differs from the species of *Coeuritrema* in minor features of a specific nature such as the size and shape of the body, size of the suckers, size and shape of the testes, ovary, vesicula seminalis and cirrus sac. In view of the foregoing, *Tremarhynchus indicus* must be considered synonymous with *Coeuritrema* and included in the latter genus on the basis of priority under the name of *Coeuritrema indicus* (Thapar). The latter species resembles *C. odhnerensis* in the shape of its body, but it is about twice in length of the latter species, and also differs in the oral sucker being larger than the ventral sucker, a feature in which it resembles *C. lyssimus*. The testes in *C. odhnerensis* are not so deeply lobed as in *C. indicus* in which they are described as divided into follicles, though Thapar's figure shows them to be deeply lobed and not separated into pieces so as to deserve the name of follicular testes, as the term is ordinarily used. The vitellaria in both these species extend a little more forwards than in *C. lyssimus*, i.e., in front of the ventral sucker as far as the intestinal bifurcation. *C. indicus* differs remarkably from the other species not only in the shape of its testes, but it also differs in the large size and position of its vesicula seminalis. The receptaculum seminis as mentioned above is presumed to be present in this species.

#### Family Spirorchidae Stunkard, 1921.

Stunkard gave a classification of this family in 1921, creating the subfamilies Spirorchinae and Hapalotreminae. In 1923 he gave a fuller account of the family assigning *Hapalotrema* Looss, 1899 and *Hapalorhynchus* Stunkard, 1922 to the subfamily Hapalotreminae and *Spirorchis* MacCallum, 1918, *Henotosoma* Stunkard, 1922 and *Haematotrema* Stunkard, 1923 to the subfamily Spirorchinae. The genera *Henotosoma* and *Haematotrema* are now held, as mentioned above, to be synonymous with the genus *Spirorchis*. Since 1923 many genera have been created and added to this family; it, therefore, appears necessary to revise the family and subfamily diagnosis, and give keys for the identification of the genera and species.

*Family diagnosis.*—Small delicate hermaphrodite blood flukes with poorly developed musculature; monostomes or distomes. Pharynx absent;

oesophagus long, surrounded by salivary gland cells, which are numerous near its posterior extremity; intestinal caeca ending blindly near posterior end, with or without forwardly directed loops at their origin; only one intestinal caecum present in *Unicaecum*. Genital opening sinistral, dorsal or ventral, about middle of body length or near hinder end. Testes two with ovary between them (*Hapalorhynchus*, *Coeuritrema*), divided into a large number of follicles forming two masses, one in front of and other behind ovary (*Hapalotrema*), one large undivided testis behind ovary (*Vasotrema*) in front of ovary (*Unicaecum*), divided into follicles all arranged in a linear series anterior to ovary (*Spirorchis*, *Plasmiorchis*) or last one or two follicles behind ovary (*Diarmostorchis*, *Spirhupalum*). Ovary usually lobed, median, to right or left side, a little behind middle, or near hinder end of body, or long and rolled in posterior part of body (*Unicaecum*); receptaculum seminis and Laurer's canal present or absent. Cirrus sac small, well developed or rarely absent (*Hapalorhynchus*); vesicula seminalis externa large; protrusible cirrus well developed in some genera. Uterus short; metraterm poorly or strongly developed; ovum large with or without polar filament or filaments, discharged singly. Vitellaria lateral and extensively developed. Excretory vesicle small, dividing almost immediately into lateral ducts. Parasites in blood of turtles.

**Type genus.**—*Spirorchis* MacCallum, 1918 (Syn. *Proparorchis* Ward, 1921).

#### Key to the subfamilies of Spirorchidae.

- |   |                       |
|---|-----------------------|
| 1. Genital pore and ovary near middle of body length.   | <i>Hapalotreminae</i> |
| Genital pore and ovary near hinder end . . . . .  | 2                     |
| 2. Testes arranged in a linear series all or except last one or two in front of ovary; two intestinal caeca present . . . . . | <i>Spirorchinae</i>   |
| Testis one continuous lobed structure and not divided into follicles; one intestinal caecum present . . . . .                 | <i>Unicaecumiinae</i> |

#### Subfamily Hapalotreminae Stunkard. 1921.

*Subfamily diagnosis.*—Spirorchidae: Genital pore sinistral, dorsal rarely ventral, near middle of body length. Testes two with ovary between them (*Hapalorhynchus*, *Coeuritrema*), divided into two masses of follicles one in front and other behind ovary (*Hapalotrema*) or only one large postovarial testis present (*Vasotrema*). Ovary lobed, faintly lobed, or entire, slightly to left side, near genital pore and middle of body length. Cirrus sac well developed, absent only in *Hapalorhynchus*, in front of ovary or anterior testis. Vesicula seminalis large, outside cirrus sac; protrusible cirrus present, absent only in *Hapalorhynchus*. Receptaculum seminis and Laurer's canal present. Vitellaria well

developed extending from intestinal bifurcation or behind ventral sucker to caudal end of caeca. Parasites of turtles

**Type genus.**—*Hapalotrema* Looss, 1899.

**Key to the genera of the subfamily Hapalotreminae**

- Testes divided into two masses, one in front of and other behind ovary . . . . . *Hapalotrema*
- Testes only two with ovary between them . . . . . 1
- 1. Cirrus sac and cirrus present . . . . . *Cheuritrema*
- Cirrus sac and cirrus absent . . . . . *Hapalorhynchus*
- Testis only one, behind ovary . . . . . *Vasotrema*

**Key to the species of the genus *Cheuritrema***

- Ventral sucker smaller than oral sucker . . . . . *C. odhnerensis*
- Ventral sucker larger than oral sucker . . . . . 1
- 1. Body narrow, pointed at hinder end; body length 3.16—3.45; vesicula seminalis large, 0.2 in length . . . . . *C. indicus*
- Body broad, somewhat rounded at hinder end; body length 1.5—2; vesicula seminalis small, 0.048—0.057 in length . . . . . *C. lyssinus*

**Subfamily Spirorchinae Stunkard, 1921.**

*Subfamily diagnosis.*—Spirorchidae: Genital pore sinistral, ventral, near hinder end of body. Testes, large in number, arranged in a linear series in the intracaecal area all or except last one or two in front of ovary. Ovary lobed, dextral or median, near genital pore and hinder end of body. Cirrus sac small, with poorly developed musculature except in *Spirhapalum* and *Plasmiorchis hardelli*. Vesicula seminalis large, outside cirrus sac, with anterior end broad and in contact with hindmost testis. Receptaculum seminis and Laurer's canal present. Vitellaria both extra and intracaecal, extending from intestinal bifurcation or about middle of oesophagus to caudal end of caeca. Parasites of turtles.

**Type genus.**—*Spirorchis* Mac Callum, 1918 (syn *Proparorchis* Ward, 1921.)

**Key to the genera of the subfamily Spirorchinae.**

- Intestinal caeca with loops at their origin, one on each side of oesophagus . . . . . *Plasmiorchis* N.G.
- Intestinal caeca without loops at their origin . . . . . 1
- 1. Testes all in front of ovary . . . . . *Spirorchis*
- One or two testes behind ovary . . . . . 2
- 2. Ventral sucker present; cirrus sac spacious . . . . . *Spirhapalum*
- Ventral sucker absent; cirrus sac poorly developed . . . . . *Diarmostorchis*

**Key to the species of the genus *Spirorchis*.**

- Testes commencing behind middle of body . . . . . 1
- Testes commencing in front of middle of body . . . . . 2



1. Testes 4 or 5 in number ; genital pore at one fourth body length from posterior end . . . . . *S. parvum*  
(Stunkard, 1922)
- Testes 10 in number ; genital pore near posterior end . . . . . *S. haematobium*  
(Stunkard, 1923)
2. Genital pore one seventh of body length from posterior end . . . . . 3
- Genital pore one fourth of body length from posterior end . . . . . 4
3. Testes larger than ovary, not distinctly separated . . . *S. innominata*
- Testes smaller than ovary, distinctly separated . . . *S. artericola*
4. Testes large, commencing immediately behind intestinal bifurcation . . . . . *S. scripta*
- Testes large, commencing some distance behind intestinal bifurcation . . . . . *S. elegans*
- Testes small, not more than one half size of ovary . . . *S. picta*

#### Key to the species of the genus *Plasmiorchis*

- Ventral sucker absent in adult, but ventral sucker area present . . . . . *P. pellucidus*
- Ventral sucker present . . . . . 1
1. Cirrus sac larger, with stout musculature . . . . . *P. hardellii*
  - Cirrus sac very small, with weak musculature as in *Spirorchis* . . . . . 2
  2. Testes 5—7 in number ; caeca straight without outgrowths . . . . . *P. orientalis*
  - Testes much larger in number ; caeca undulating near posterior end and with small outgrowths . . . . . *P. obscurum*

#### Subfamily Unicaecuminae, new subfamily

*Subfamily diagnosis.*—Spirorchida :—Only one intestinal caecum present, the other atrophied and absent. Testis one continuous lobed structure, and not divided into separate follicles; vas deferens arises from anterior end of testis and not from posterior extremity as in Spirorchinae; vesicula seminalis very long, spirally coiled and parallel to testis throughout length of body. Ovary long and coiled in posterior part of body. Parasitism of turtles.

**Type genus.**—*Unicaecum* Stunkard, 1927.

#### Discussion on the Relationships of the Families of Blood Flukes.

The relationships of the Spirorchidae with the Schistosomatidae have been discussed already (1933). The subfamily Hapalotreminae represents the ancestral blood flukes, from which have been evolved along one line the Schistosomatidae, and along another the degenerate suckerless blood flukes of the families Aporocotylidae and Sanguinicolidae. It is this latter part of the theme which we discuss further in this paper.

The highly interesting genera of blood flukes *Aporocotyle*, *Psettarium*, *Deontacylix* and *Sanguinicola* are unique among the Digenetic trematodes in having lost both the suckers. *Aporocotyle* was discovered by Odhner in 1900 as an ectoparasite from the gills of flounder. It was later announced by him in 1911 as a blood fluke. *Sanguinicola* was discovered by Plehn in 1905 as an endoparasitic Turbellarian, and included by her in a new family Rhynchostomida. Later in 1908 she considered it as a monozoic Cestode. Lühe in 1910 created for it a separate order of Cestoda, Rhynchostomida M. Plehn. Odhner in 1911 after comparing it fully with *Aporocotyle* and *Haplotrema* recognised its true nature as a Digenetic trematode. He also reported in this paper that the suckerless *Deontacylix ovalis* Linton from the intestine of a West Indies fish may be a blood fluke, which in reality is a relation of *Sanguinicola*, and in regard to the structure of the gut occupies an intermediate position between it and *Aporocotyle*. In 1912 Odhner created for the blood parasites of *Aporocotyle*-*Sanguinicola* series a new family Aporocotylidae, which was accepted and defined by Stunkard in 1923. Woodland in 1923, on the basis of his erroneous description of the genital organs of *Sanguinicola* in the blood of Siluroids from Sudan, agreed with Plehn's first idea of regarding that genus as an aberrant and much modified Turbellarian, and denied entirely its Malacocotylean affinities. Odhner in 1924 pertinently corrected this idea after giving a correct description of the African species, which he named *S. chalmersi*. The morphology and development of the European species of *Sanguinicola* have been recently investigated by Ejsmont in 1926. Poche in 1925 and Fuhrmann in 1930 accepted the family Aporocotylidae for *Aporocotyle* and *Deontacylix* and the family Sanguinicolidae for *Sanguinicola* in the Digenea. The latter family was created by Graff in 1907, when *Sanguinicola* was included in the Turbellaria. \*Rasin in 1929 assigned a new genus *Janickia* to the Sanguinicolidae, to which has also been added by Van Cleave and Mueller in 1932 a new species *Sanguinicola occidentalis* obtained from the heart of *Stixostedion vitreum* from Oneida Lake. Layman in 1930 described a new species *Aporocotyle odhneri* in blood of *Spheroides borealis* from the Sea of Japan. In 1929 Goto and Ozaki discovered in the intestine of a puffer, *Plehnia japonica* which they called *Psettarium japonica* in 1930. The latter species, which is considered to be closely related to *Deontacylix* appears to be a blood fluke. As its discoverers had apparently recourse to only one specimen for description, its true habitat in mesenteric blood vessels might have escaped their notice. Their statement that no blood corpuscles were found in the intestinal contents of the parasite does not seem to be a valid argument for denying its haematic abode, as Plehn in her first description had also stated that blood corpuscles were never found in the gut of *Sanguinicola*.

\* Biol. Spisy. Brno. 8, XVI, 1929.

From the account of morphology it is clear that we have a descending series in the evolution of the alimentary system of the four suckerless trematodes hitherto known, *Aporocotyle*—*Psettarium*—*Deontacylix*—*Sanguinicola*. In all these genera the gut is of the basic H-shaped type. In *Aporocotyle* the oesophagus is of about the same length as in the Spirorchidae and Scistosomatidae; it is also surrounded as in these families by the salivary gland cells. The intestinal caeca are also of the same length and reach near the hind end. The only point of difference, however, is the presence of anterior blind sacs in *Aporocotyle*. The intestinal caeca of *Plasmiorchis* have got forwardly directed loops at their origin exactly in a similar position to that of the anterior blind sacs of the gut of the latter genus. There is a peculiar similarity of the gut in this feature in the two genera. We may say that the formation of loops by the caeca at their origin as in *Plasmiorchis* provides an appropriate condition for the origin of the blind sacs at the anterior ends of the caeca of *Aporocotyle*. From this it follows that *Plasmiorchis* is related to the ancestral blood fluke from which *Aporocotyle* is evolved. It appears that the evolution in the blood flukes, as in the rest of the Digenea, has taken place by mutations as displayed by certain basic tendencies. Just as an ancestral form like *Plasmiorchis* came into existence with loops at the origin of the caeca to provide for an increased absorptive surface for food, another form closely related to it arose in the Hapalotreminae having two limbs of the loop fused, as it were, to form the anterior blind sac of one side of the gut. From the gut of *Aporocotyle* can be derived that of *Psettarium* in which the oesophagus is long and opens in the centre of the H-shaped intestine. The anterior caeca are small, *i.e.*, about one third as long as the oesophagus, but the posterior caeca are much smaller than those of *Aporocotyle*, terminating much in front of the hinder end. From *Aporocotyle* onwards there is a great tendency in this series of genera towards a reduction in the length of the intestinal caeca and from *Psettarium* onwards there is also a tendency towards a greater development of the anterior horns. This has culminated in the extremely small H-shaped gut of *Sanguinicola*, in which both the anterior and posterior horns are of nearly equal size. *Deontacylix*, in this respect, occupies an intermediate position between *Psettarium* and *Sanguinicola*. It has posterior caeca much smaller and anterior caeca much larger than those of *Psettarium*, but they are both of nearly equal size as in *Sanguinicola*, though much larger than in the latter genus. Goto and Ozaki do not mention the presence of salivary gland cells in *Psettarium*, but we presume that they are present in this genus also. In *Sanguinicola chalmersi* the lobes of the H-shaped gut have lost their separate entity and the gut has consequently taken an irregular shape of the Rhabdocoele type. The mouth opening is extremely small in these blood-sucking genera.

There is a close similarity in the genital organs of these four genera. The testes in all of them are divided into a large number of follicles. It has been suggested in a previous paper that the ancestral blood fluke possessed two testes with the ovary between them like *Coeuritrema* and that the presence of a large number of testes in front of the ovary, which lies near the hind end as in the Spirorchinae and the Aporocotylidae, is a secondary condition evolved from the condition in the Hapalotreminae, in which the anterior testicular mass developed preponderantly, so that the ovary with its associated ducts and the genital pore came to lie near the hind end, whilst the posterior testis not possibly divided into follicles became entirely suppressed. In *Aporocotyle* the testes, large in number, occupy irregularly the entire intracaecal region between the intestinal bifurcation and the ovary; in the Spirorchinae they occupy the same position with this difference that their number is smaller and arrangement regular in a linear series. The former condition is primitive and the latter secondary. We are inclined to believe that *Aporocotyle* represents the origin of one-side branch and Spirorchinae as the other of the main stem represented by the common ancestor, in which the *Aporocotyle* arrangement of testes and ovary was present. In *Psettarium* and *Deontacylix* the testes lie both outside and inside the caeca, occupying nearly the entire space available, between the intestinal bifurcation and the ovary, on account of reduction in length of the caeca and their approachment towards each other near their origin. In *Sanguinicola*, however, their arrangement is somewhat regular in a double row, behind the gut and in front of the ovary, but this is obviously a departure from the irregular arrangement of *Aporocotyle* type along another direction from that, shown by the testes of *Psettarium* and *Deontacylix*. The shape of the ovary varies in different genera or even in different species of the same genus and should not be considered of much importance from the point of view of these relationships. It is spherical or ovoid in *Aporocotyle*, slightly lobed in *Deontacylix*, much lobed in several species of *Plasmiorchis* and *Spirorchis*, ramified or aciniform in *Psettarium* and H-shaped in *Sanguinicola*.

The genital pore or separate male and female openings in the suckerless genera lie dorsal to the left side (dorsal and nearly median in *Sanguinicola*) near posterior end. In the Hapalotreminae also the genital pore lies dorsal to the left side, though near the middle of the body length. This also supports the view mentioned above about the evolution of these forms from the Hapalotreminae. The Spirorchinae, in which the genital pore is ventral near the posterior end, represents obviously another line from the same common ancestor. The genus *Aporocotyle* among the genera of its own line stands closer to that ancestor, in that it has one opening for both male and female ducts, which lies in front of the ovary; whereas the other genera show a specialized condition in that the male and female openings are separate,

a condition which is not without its parallel in the Digenea. The cirrus sac in *Aporocotyle* is also fairly well developed, resembling that of the Haplotremata. Though it is smaller in *Psettarium* and *Deontacylix*, it is conical and bent as in the latter subfamily. A large vesicula seminalis outside the cirrus sac similar to that of the Spirorchidae is present in *Deontacylix*. The uterus in *Aporocotyle* and *Psettarium* is much larger than in the latter family consisting of a number of convolutions and containing a large number of ova. In *Deontacylix* it is much larger and filled with numerous ova. As in the Schistosomatidae the genus *Schistosoma* has secondarily developed a uterus containing a large number of ova, whereas its ancestors *Bilharziella*, *Ornithobilharzia*, *Austroilharzia* and *Heterobilharzia* have a very small uterus containing only one ovum, in the same way *Aporocotyle*, *Psettarium* and *Deontacylix* have secondarily acquired a large uterus. In this respect *Sanguinicola* with a very small uterus containing only one ovum shows the primitive condition. The metraterm is well developed in *Aporocotyle* as in many Haplotremata. The vitellaria are extensively developed in the latter genus as in the Spirorchidae. In *Psettarium* they are still more extensive, occupying the entire ventral surface of the body from the anterior end to the ovary. In *Sanguinicola* they are also extensive; in *S. chalmersi* they extend posteriorly even behind the ovary. There is only one vitelline duct present in all these genera except *Sanguinicola occidentalis* the left one having disappeared as pointed out by Odhner.

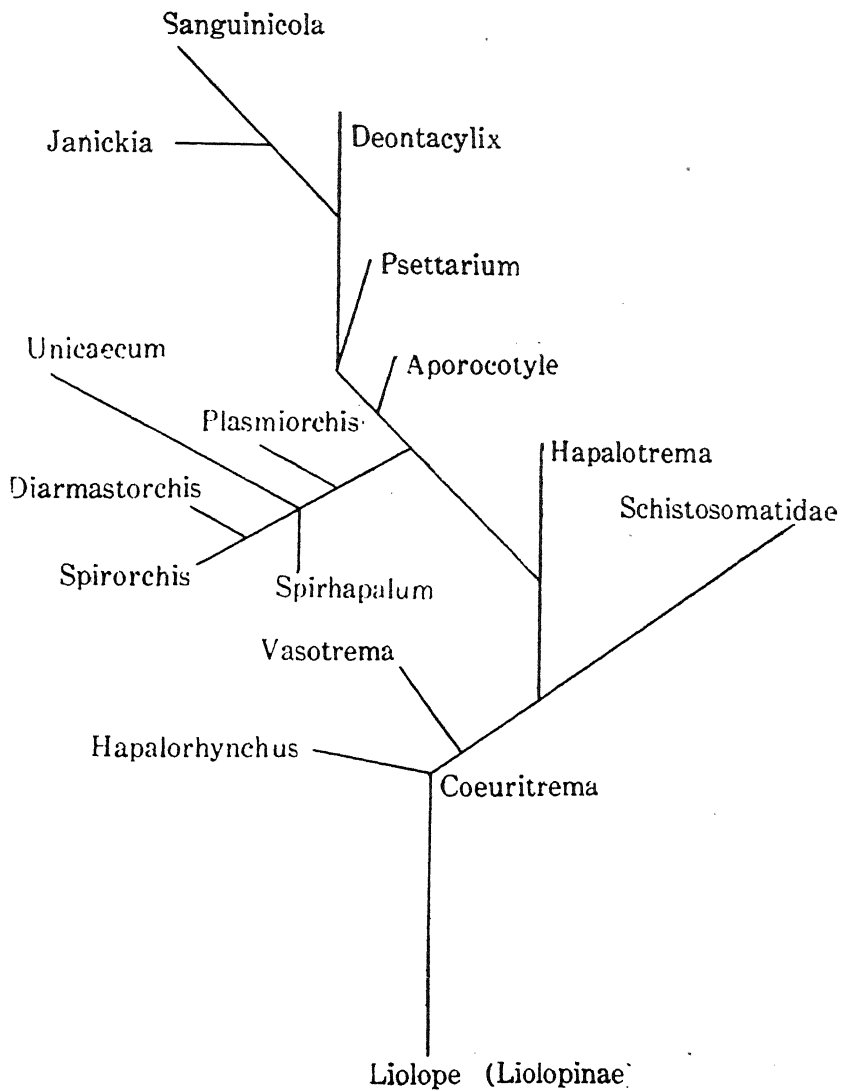
In the excretory, nervous and muscular systems also there is a substantial agreement not only between these four genera but also between them and the Spirorchidae. In the scheme of relationships of the blood-fluke families it appears certain, as shown above, that *Aporocotyle* stands near the Spirorchidae, and represents a close relation of the ancestor, from which are evolved along one line its closely related genera *Psettarium*, *Deontacylix* and *Sanguinicola* and along another the subfamily Spirorchinae. The genus *Unicaecum* has arisen as an aberrant branch from the latter subfamily, and we accordingly, include it in a new subfamily. *Aporocotyle*, *Psettarium* and *Deontacylix* are assigned to the family Aporocotylidae.

#### EXPLANATION OF THE PLATES.

- Fig. 1. Ventral view of *Plasmiorchis orientalis*.  
 Fig. 2. Dorsal view of *P. pellucidus*.  
 Fig. 3. Ventral view of *P. hardellii*.

#### Microphotographs

- Fig. 4. *P. orientalis*. Leitz Eyepiece X; Leitz Objective I.  
 Fig. 5. *P. pellucidus*. Leitz Eyepiece X; Leitz Objective I.



Tree indicating the probable phylogeny of the blood flukes

- Fig. 6. *P. hardellii*. Zeiss Eyepiece X; Leitz Objective I.  
 Fig. 7. *P. obscurum*. Leitz Eyepiece X; Leitz Objective I.  
 Fig. 8. Immature specimen of *P. orientalis*. Leitz Eyepiece X; Leitz Objective I.  
 Fig. 9. T. s. of *P. orientalis* in the region of ventral sucker. Leitz Eyepiece 3; Leitz Objective 45 X.  
 Fig. 10. Hinder part of body of *P. pellucidus* Zeiss Eyepiece X; Leitz Objective 3-10 X.  
 Fig. 11. Hinder part of body of *P. hardellii*. Leitz Eyepiece 3; Leitz Objective 3-10 X.  
 Fig. 12. Part of t. s. of *P. hardellii* in the region of cirrus sac. Leitz Eyepiece 3; Leitz Objective 3-10 X.  
 Fig. 13. T. s. of *P. hardellii* in the region of metraterm, cirrus sac and genital opening. Leitz Eyepiece 3; Leitz Objective I.

#### LETTERING.

a. l. i. c., anterior loop of intestinal caecum; c. s., cirrus sac; e. v., excretory vesicle; g. l. i. c., genital loop of intestinal caecum; g. o., genital opening; g. v., glandular vesicle; i. c., intestinal caecum; m., metraterm; o. s., oral sucker; oes., oesophagus; oes. v., oesophageal vesicle; ov., ovary; r. s., receptaculum seminis; t., testis; ut. uterus; v. s., ventral sucker; v. r., vitelline reservoir; v. s. a., ventral sucker area; v. sm., vesicula seminalis; vit., vitellaria.

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**Errata in Part I Published in *Bull Acad. Sc. U. P., Allahabad*. Vol 2,****No. 4, May 1933:—**

Page 207, line 19 for "distel" read distal.

Page 209, line 2 from bottom add between "right intestinal caecum" and "by the intervening metraterm" the following, -separated from the left caecum.

Page 213, line 3 after *Henotosoma* Stunkard for "1923" read 1922.

Page 213, line 9 for "*Haemato rema*" read Hapalotrema.

Page 213, line 22 for "Proparorchidae" read Proparorchis.

Page 21, line 12 for "Zoolopathologica" read Zoopathologica.

Page 221, line 17 for "Pein" read ein.

Page 221, line 19 for "(1921)" read (1912)

Plate I

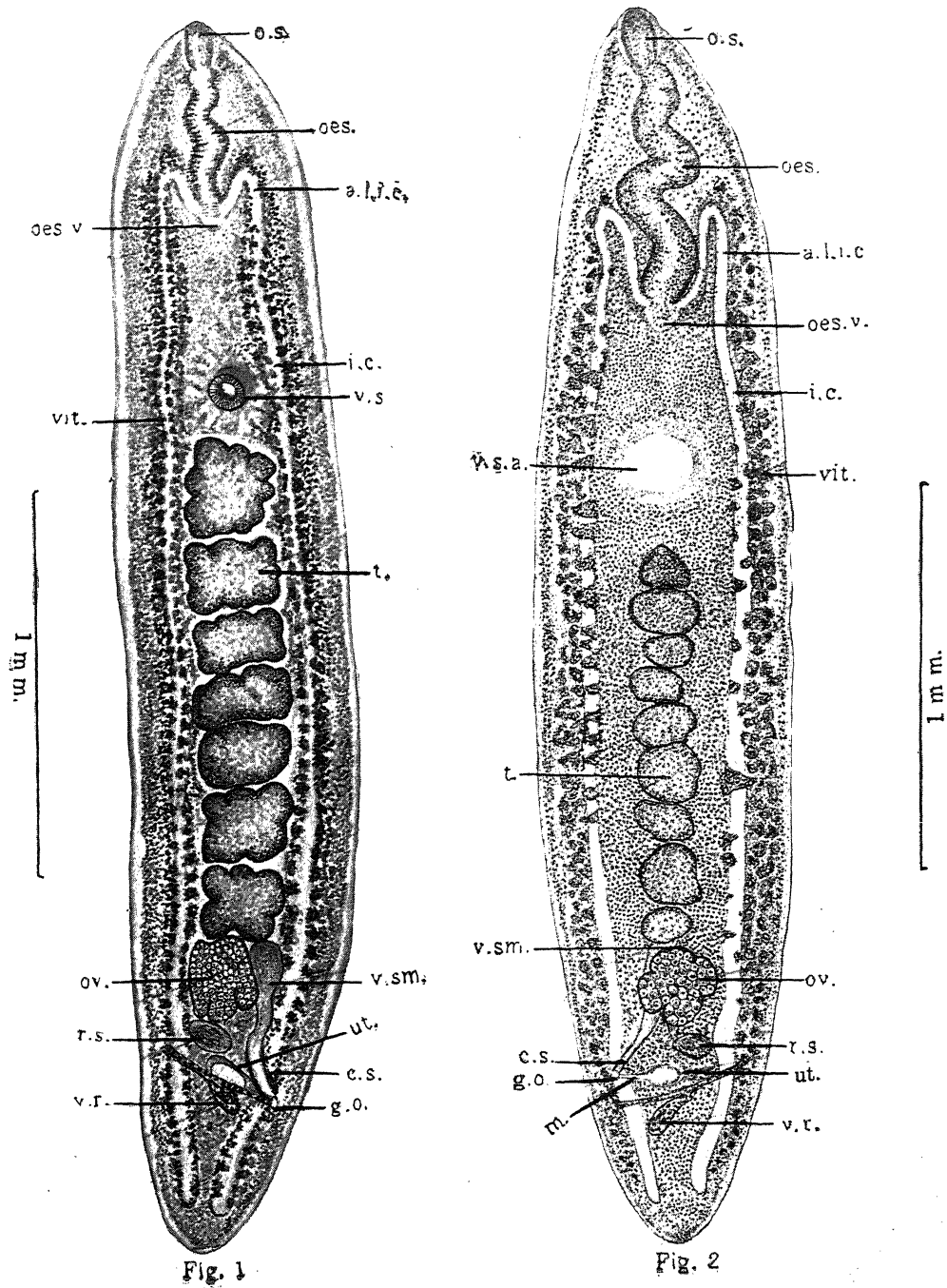




Plate II

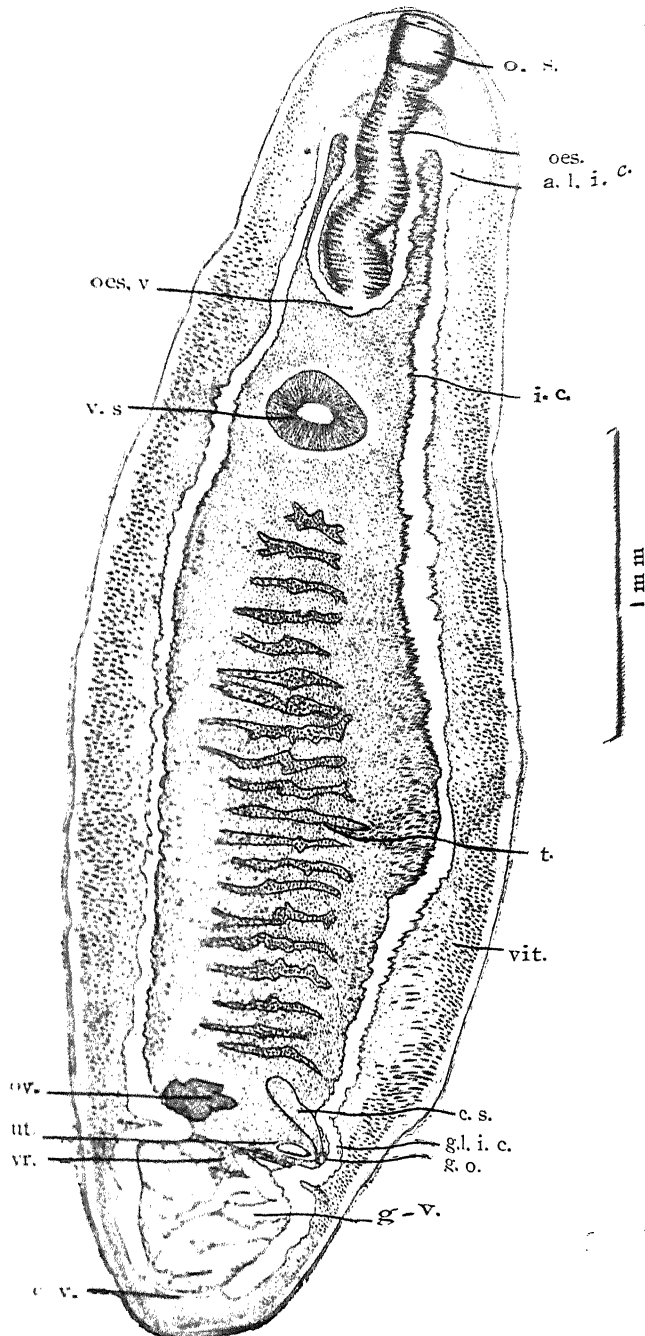


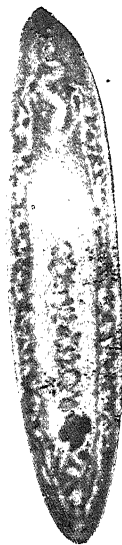
Fig. 3



Plate III



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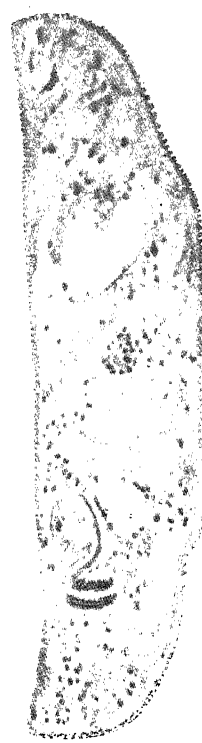
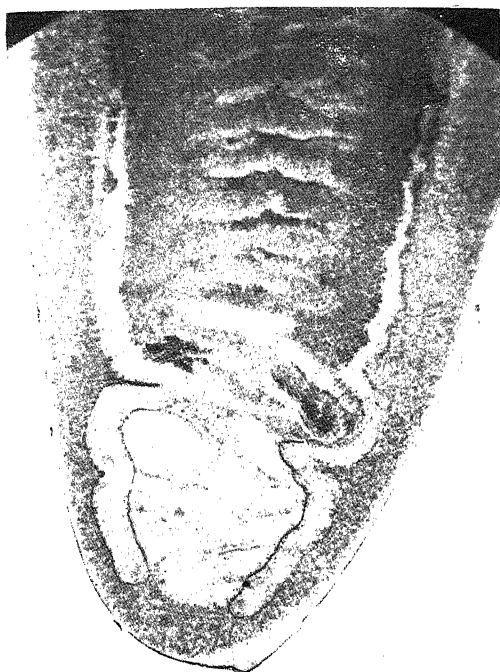
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Plate IV







# ON THE ABSORPTION SPECTRUM OF NITROGEN MONOXIDE IN THE SCHUMANN REGION

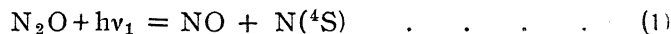
By P. K. SEN-GUPTA

DEPARTMENT OF PHYSICS, ALLAHABAD UNIVERSITY

Communicated by Prof. M. N. Saha

Received December 14, 1933

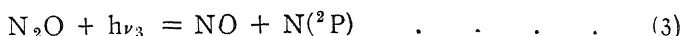
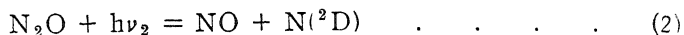
The absorption spectrum of Nitrogen Monoxide was studied by Leifson<sup>1</sup> in the Schumann region and by Datta<sup>2</sup> in the quartz region. Leifson used a vacuum grating spectrograph and absorption cells with fluorite windows and worked chiefly between  $\lambda 2400$  and  $\lambda 1240$ . The absorption column was 1.5 cms. in length with  $N_2O$  at atmospheric pressure. He found two continuous absorption bands, the first extending from  $\lambda 2000$  to  $\lambda 1680$ , and the second from  $\lambda 1550$  to the limit of fluorite. But no explanation of these cuts were offered. Six years later Wulf and Melvin<sup>3</sup> performed some irradiation experiments with  $N_2O$  and showed that it is photochemically decomposed by light of wavelength  $\lambda 2300$  into NO and N. In 1932 Datta again studied the absorption of  $N_2O$  in the quartz region, and tried to interpret his results from thermochemical data. He found  $N_2O$  to absorb light continuously from a long wavelength limit and traced it with the aid of some microphotograms to  $\lambda 2750$  which is much further on the long wavelength side than Wulf and Melvin's limit. Ascribing the beginning of absorption at  $\lambda 2750$  to the photo-dissociation of  $N_2O$  into NO and  $N(^4S)$ , as given in



Datta calculated the heat of dissociation of  $N_2$  with the aid of some thermochemical equations and obtained a value of 8.7 volts which agreed well with some other correct determinations of  $D_{N_2}$ . This proved the validity of assuming a process like (1).

At first sight this process seems to have no connection with Leifson's absorptions at  $\lambda 2000$  and  $\lambda 1550$ . In this connection attention may be drawn to the cases of  $SO_3^4$  and other higher oxides<sup>5</sup> and sulphides<sup>6</sup> and other

compounds in which a first absorption is generally followed by retransmitted patches of light with subsequent absorptions which correspond to liberation of atoms in metastable states. With  $\text{N}_2\text{O}$  a similar behaviour should be expected with a second and third setting of absorption according to the following equations,



where  $^2\text{D}$  and  $^2\text{P}$  are the metastable states of nitrogen. Taking Compton and Boyce's<sup>7</sup> value of  $^4\text{S}-^2\text{D}$  of nitrogen, Datta identified the second of Leifson's limits at  $\lambda$  1550 as indicating the decomposition of  $\text{N}_2\text{O}$  into NO and N in the  $^2\text{D}$ -state, on the grounds that with the aid of microphotograms  $\lambda$  1550 could be extended to  $\lambda$  1840 which is the expected place. The following work in the Schumann region was performed with a view to verify this point.

### EXPERIMENT

I have used a fluorite prism spectrograph designed according to our own directions. The spectrograph has strong light gathering powers and the region  $\lambda$  2200 to  $\lambda$  1300 comes on a plate 10 cms. long. It is specially designed for absorption work in the Schumann region. The absorption chamber which was of glass was separate and could be sealed to the end of the spectrograph carrying the fluorite window. The spectrograph and the absorption chamber were evacuated separately by common Holweck molecular pump and in the line a discharge tube was connected to test the vacuum. To the other end of the absorption chamber a hydrogen tube (run by a 2KW transformer) with a fluorite window was joined by means of a wide bore pressure tube. Thus the fluorite windows of the hydrogen tube and the spectrograph were common to the absorption chamber also. The spectrum of hydrogen served as a continuous source of light up to  $\lambda$  1600 after which the secondary emission spectrum of hydrogen appeared extending to the limit of fluorite. An idea of the vacuum in the chamber could be formed from the nature of the discharge in a vacuum tube attached to the spectrograph which was run by an induction coil.

It was found by trial that the length of the absorption chamber about 30 cms.) was too long for the present purpose, and therefore a separate absorption cell having a length of only 10 cms. had to be inserted into

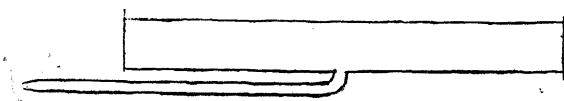


Fig. 1

the main chamber. This cell carried as usual fluorite windows at the ends and

was also provided with a bent side tube so as to facilitate insertion in the main chamber. After the cell was filled with  $N_2O$  dried with  $P_2O_5$  at the requisite pressure the extreme end was sealed off. Fig. 2 shows the main absorption chamber and the subsidiary absorption cell in position. A

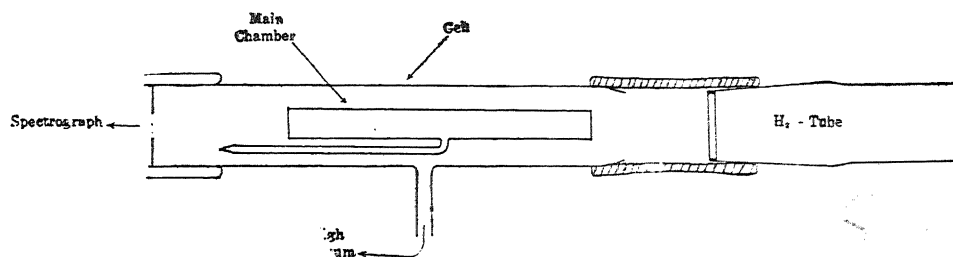


Fig. 2

series of different pressures were used ranging from 0.05 cm. to 20 cms. and photographs taken on Schumann plates.

The development of the absorption spectrum was as follows. At very low pressures light was cut off at about  $\lambda$  1850 and reappeared at about  $\lambda$  1700 and was again cut off at about  $\lambda$  1580. These absorptions were rather sharp in comparison to Datta's absorption in the quartz region. With increasing pressures the retransmitted patch of light from  $\lambda$  1700 to  $\lambda$  1580 vanished and only the absorption at  $\lambda$  1850 was visible. At still higher pressures the absorption at  $\lambda$  1850 began to extend towards longer wavelengths finally extending beyond  $\lambda$  2200. This was due to the fact that owing to heavy absorption, the retransmitted patches are wiped out.

#### DISCUSSION

The first beginning of absorption was found by Datta to correspond to  $\lambda$  2750. Now according to the second and third processes  $h\nu_1 - h\nu_2$  and  $h\nu_2 - h\nu_3$  should give approximate values of  $^4S - ^2D$  and  $^2D - ^2P$  of nitrogen, which, from the work of Compton and Boyce (loc. cit.) on the classification of arc spectrum of nitrogen are known to be 2.37 and 1.19 volts respectively. Since  $h\nu_1$  is equal to the energy corresponding to  $\lambda$  2750, that is, 4.53 volts,  $h\nu_2$  should be 6.9 volts corresponding to  $\lambda$  1800 and  $h\nu_3$  should be 8.1 volts corresponding to  $\lambda$  1540. In the present experiment the first beginning of absorption at  $\lambda$  1850 and the second at  $\lambda$  1580 no doubt correspond to  $h\nu_2$  and  $h\nu_3$  respectively. The experimental value, therefore, are—

$$h\nu_{2750} - h\nu_{1850} = ^4S - ^2D \text{ of N} = 2.17 \text{ volts,}$$

$$h\nu_{1850} - h\nu_{1580} = ^2D - ^2P \text{ of N} = 1.13 \text{ volts.}$$

In some of these cases it seems that molecular spectra yield low values of the difference of atomic terms—this phenomenon is yet not satisfactorily

explained. That the difference might not be the same as those obtained by the

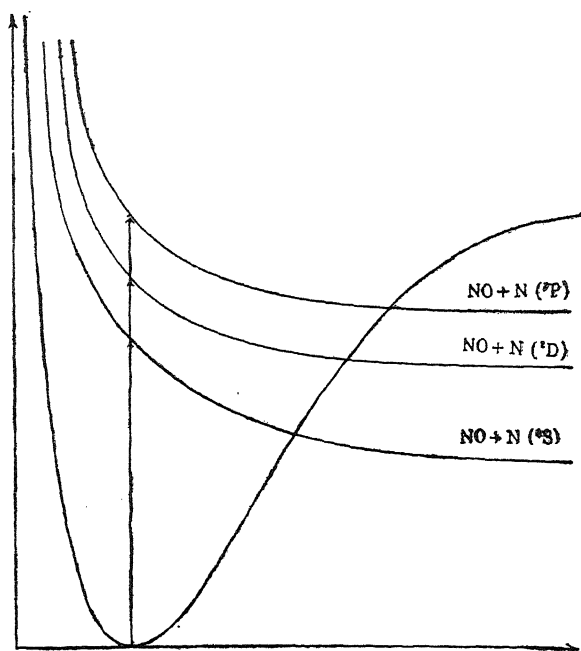


Fig. 3

arc spectrum could be understood from the adjoining potential energy diagram, since we do not know the nature of the upper curves. This point has been discussed in the paper on the sulphides of zinc, cadmium and mercury.<sup>8</sup>

Now it has already been mentioned that Datta identifies Leifson's absorption at  $\lambda 1550$  as due to the interaction of a nitrogen atom in the  $^2D$  state with NO. His argument is based on the grounds that since the first absorption at  $\lambda 2750$  is extended the second should also be extended; and as

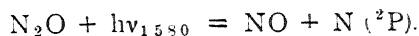
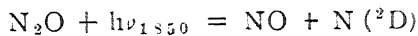
Leifson has no doubt given the values of complete extinction of light at  $\lambda 1550$ , this could be traced to  $\lambda 1840$  nearly with the aid of microphotograms. It might be remarked here that from the appearance of an extended absorption as a first cut one cannot predict that the subsequent absorptions will be extended too. In the present experiment it has been found that the absorptions in the Schumann region are not at all extended and therefore it is highly improbable for a shift of about  $290 \text{ \AA}$  to take place by microphotograms. Leifson's absorption at  $\lambda 1550$  obviously corresponds to an interaction of NO with a nitrogen atom in  $^2P$  state, and not  $^2D$  state as Datta holds. Leifson used a high pressure in his absorption cell, and this most probably accounts for the absence of the second absorption at  $\lambda 1850$ . We generally come across such peculiar behaviour of gases at different pressures. It appears that the absorption at  $\lambda 2000$  obtained by Leifson is the same as  $\lambda 1850$  shifted towards longer wavelengths due to high pressure.

In conclusion the author gratefully acknowledges the valuable guidance rendered by Prof. M. N. Saha, D.Sc., F.R.S.

#### SUMMARY

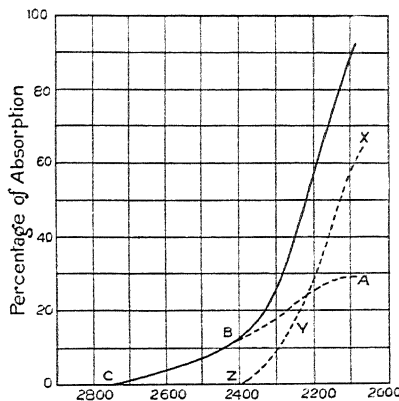
Absorption experiments with  $N_2O$  has been performed in the Schumann region at different pressures. It has been found that light is continuously cut off at  $\lambda 1850$ , reappears at about  $\lambda 1700$  and is again cut off at  $\lambda 1580$ . It is

suggested that the foregoing absorptions are due to photochemical dissociation of  $N_2O$  into NO and nitrogen in the metastable states according to the following—



#### SUPPLEMENT ADDED IN PROOF

On. of Datta's<sup>2</sup> curves for  $N_2O$  in the quartz region has been reproduced here. The point C corresponds to the long wave beginning of absorption which marks the photochemical dissociation of  $N_2O$  into NO and  $N(^4S)$ . But the curve has rather a peculiar shape. It is not smooth, but has a discontinuity at the point B. The author<sup>9</sup> has shown elsewhere that a discontinuity or Kink at any point of the absorption curve has a special significance. It has been shown that generally the main curve is not a single one but composed of two curves which have been drawn for  $N_2O$  here by dotted lines. In the figure these are ABC and XYZ which are quite distinct from each other.



What is the process of dissociation of  $N_2O$  when we get two beginnings of absorption corresponding to two curves? This means that there are two processes.

Light is thrown on this problem from a scrutiny of the structure of  $N_2O$ . Various experiments on Dielectric constant have shown that while some of the investigators have found the electric moment as zero, others find a small positive value for it. The following models of  $N_2O$  are possible—

- (1)  $N-O-N$  :—The bonds may be single or multiple. No electric moment is expected for such a model on account of symmetry.
- (2)  $N \equiv N=O$  :—This model will show electric moment.
- (3)  $\begin{array}{c} O \\ \diagup \quad \diagdown \\ N \quad N \end{array}$  :—The O—N bonds are similar, but electric moment is possible.

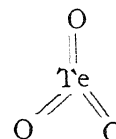
The study of absorption spectrum of  $N_2O$  definitely rules out the symmetrical rod model for it, as in this case there is only one type of bond to be severed. Corresponding to this splitting there will be only one absorption curve. The figure shows that there are two beginnings of absorption which

can only be explained if we suppose that there are two types of bonds present in  $N_2O$  having different energies, as in model 2 or 3.

The curve XYZ may be attributed to a splitting of the N—O bond, the energy of which is given by Z, that is,  $\lambda 2400 \equiv 5.2$  volts nearly. And ABC will correspond to the breaking of the N—N bond with energy given by C, that is,  $\lambda 2750 \equiv 4.5$  volts.

In  $CH_2Cl_2$ , by splitting the absorption curves N. K. Saha<sup>10</sup> first obtained evidence of the rupture of two types of bonds C—H and C—Cl, the difference in energy of which agreed remarkably well with that obtained thermo-chemically.

In  $TeO_3$  the structure is of the type shown from which we can expect only one smooth curve and no sign of kinks, as there is only one type of bond to be ruptured. This is borne out by experiment.<sup>5</sup>



In a recent paper,<sup>11</sup> Prof. Watson and his co-workers have given an account of the determination of the electric moment of  $N_2O$  and some other compounds. They have found a definite value for its electric moment which could not be decided by earlier measurements. The conclusion, they have drawn on the strength of their measurements regarding the structure of  $N_2O$  agrees with that arrived at in the present paper independently.

### References

1. Leifson, *Ast. J.*, Vol. 67, p. 73, 1926.
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# ON THE ABSORPTION SPECTRA OF THE MONOXIDES OF THE ALKALINE EARTH METALS

BY P. K. SEN-GUPTA.

PHYSICS DEPARTMENT, UNIVERSITY OF ALLAHABAD

Communicated by Prof. M. N. Saha.

Received March 5, 1934

It has already been shown by the author in several papers that the action of light on diatomic compounds of the oxide group is similar to that of the Alkali Halides, first investigated by Franck<sup>1</sup> and his co-workers. If we represent any of these compounds by MX where M is a divalent metal and X an element of the oxygen group, then on illumination of the vapour of the oxide by light of suitable frequency, the molecule is dissociated into its constituent atoms. The energy involved may be calculated from the beginning of the continuous absorption. There may be further cuts corresponding to the dissociation of the molecule into its excited atoms.<sup>2</sup>

The emission band spectra of the Monoxides of the Alkaline Earth metals were studied by Mecke,<sup>3</sup> Mahanti<sup>4</sup> and others, who have shown that the bands are due to a  $^1\Sigma \rightarrow ^1\Sigma$  transition. The present work deals with the absorption spectra of these oxides.

In a previous paper it has been shown that the oxides in the vapour state are polar compounds being formed of the ions  $M^{++}$  and  $X^{--}$  and it appeared from the interpretation of the absorption spectrum that light pulse as soon as it begins to be absorbed drives out both the electrons in  $X^{--}$  to  $M^{++}$  thus causing the oxide to be split up into M and X. In the present investigation I have tried to find whether the absorption by CaO, SrO and BaO can be interpreted in the same way.

## EXPERIMENT

It is well known that all these oxides are highly refractory. Mellor in his Treatise of Inorganic Chemistry writes, "..... W. R. Mott estimated that the boiling points of CaO about 3400°C and those of SrO and BaO occur respectively at 3000°C and 2000°C ...". For absorption it is not necessary that we should have vapour at sufficiently high pressure almost equal to that of the atmosphere, below which absorption can be detected. In fact, Claassen and Veenemans<sup>5</sup> have been able to determine the vapour pressures of the compounds between 1600—1750°K for CaO, 1500—1650°K for SrO, and 1200—1500°K for BaO, the temperatures being taken above the absolute zero. It has



been pointed out by many workers in the field that with very high temperatures the beginning of absorption is shifted towards longer wavelengths. To avoid errors due to shift, therefore, it was thought advisable to stick to the ranges given by Claassen and Veenemans as nearly as possible

The substances were vaporised in the vacuum graphite furnace of our laboratory<sup>11</sup> in the presence of nitrogen at atmospheric pressure. The column of vapour was nearly 15 cms. long in each case. Silica tubes were used to hold the substances. The source of light was a Hydrogen discharge tube run by a 2 KW transformer, and the copper arc was used for comparison. Photographs were taken by means of an  $E_3$  quartz spectrograph, on Schumann plates. To test the visible part of the spectrum a constant deviation spectrograph was used with either Process plates or Panchromatic plates, where necessary. Exposures were of the order of 2 to 3 minutes.

### RESULTS

In every case the spectrum was found to be continuously cut off from a long wavelength limit, and there was no trace of bands. To locate the beginning of the continuous absorption microphotograms were taken by the microphotometer belonging to the Physics Department, Muslim University, Aligarh. Here I must mention that I am very thankful to Dr. R. K. Asundi Reader in Physics there, for taking the microphotograms for me. On the same plate two exposures were given, that is, once the spot of light was allowed to run along the continuous spectrum and then the absorption spectrum.

Taking the ordinates of the continuous spectrum as 100 the percentage

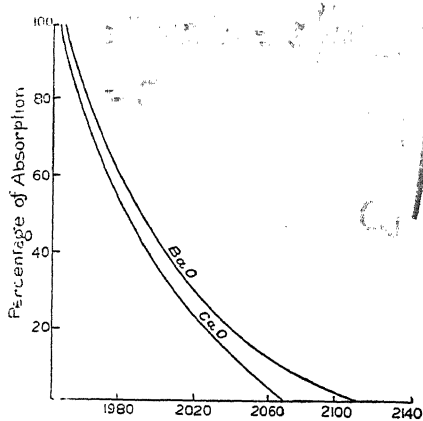


Fig. 1

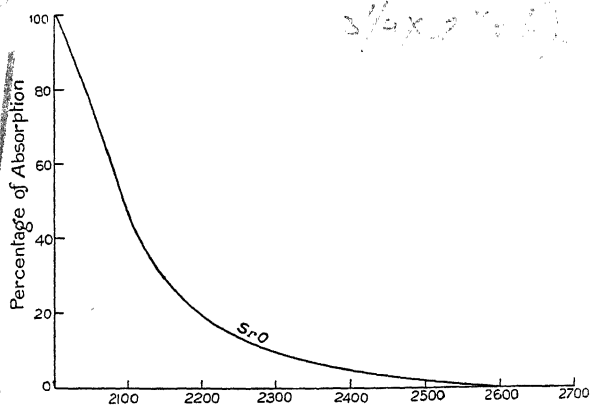


Fig. 2

of absorption was calculated from the absorption curves, and plotted with wavelength as abscissa. The point where such a curve meets the abscissa gives the beginning of absorption. From Figs. 1 and 2 we see that for CaO it is  $\lambda$  2070, for BaO  $\lambda$  2110, and for SrO  $\lambda$  2600 Å.

## CALCULATIONS

Taking  $R$  as the heat of dissociation of  $MX$  into  $M$  and  $X$  we get the following results from the Born Cycle, thermochemically.

Here  $L_{MX}$  is the Latent Heat of vaporisation of  $[MX]$ , and  $L_M$  that of  $[M]$   $D_{X_2}$  is the Heat of Dissociation of  $X_2$ .

Therefore,

$$R + L_{MX} = Q + \frac{1}{2}D_{X_2} + L_M$$

$$R = Q + \frac{1}{2}D_{X_2} + L_M - L_{MX}$$

The following table gives the values of the different quantities, the calculated values of  $R$  and the long wave limits of absorption from the graphs. The values of  $L_{MX}$  have been taken from Claassen and Veenemans' paper, and other data from Landolt and Börnstein and other tables.

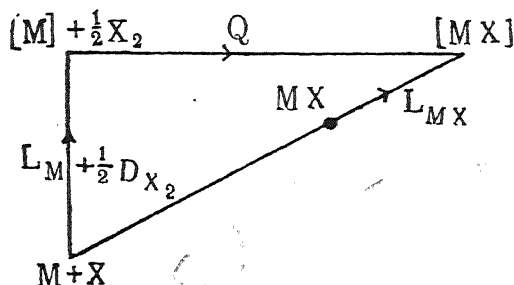


Table I

MX	$Q$ k. cal.	$\frac{1}{2}D_{X_2}$ k. cal.	$L_M$ k. cal.	$L_{MX}$ k. cal.	$R$ k. cal.	$h\nu$
CaO	145.0	64	44.2	120	133.0	$\lambda$ 2070
SrO	141.0	64	32.5	140	98.2	$\lambda$ 2600
BaO	125.9	64	32.4	90	132.3	$\lambda$ 2110

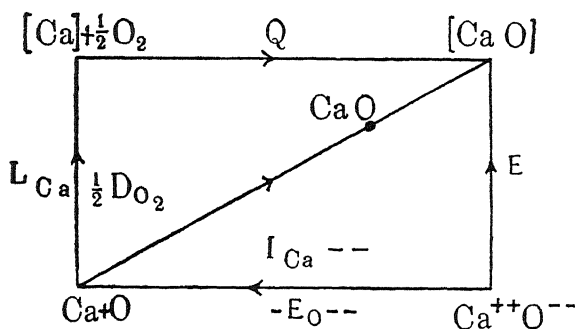
Now  $R = \frac{Nh\nu}{J}$ , and we see from Table I that there is fair agreement in the cases of CaO and BaO, but the wide divergence in the case of SrO leads one to doubt the correctness of the estimation of the latent heat of SrO. If we calculate indirectly from the long wave beginning of absorption, we find that  $L_{SrO} = 128$  k. cal.

## DISCUSSION

The appearance of the continuous absorption at the end of the spectrum in each case shows that the long wave limit of absorption is due to a transition from a firmly bound ground state to an unstable excited state. In such a case when no bands are present the binding in the ground state is supposed

to be of the ionic type. In the solid state CaO, SrO and BaO form ionic lattices of the type of NaCl. With this view in mind, therefore, we should assign such an electron structure to a compound like CaO which is also consistent with its diamagnetic behaviour. This is afforded with the structure  $\text{Ca}^{++}\text{O}^{--}$ . Here  $\text{Ca}^{++}$  and  $\text{O}^{--}$  both have the configuration  $[\text{Ar}]p^6$ , where  $[\text{Ar}]$  represents an inert gas core. The  $^1\text{S}_0$  state which results from this configuration contributes nothing to magnetism.

Taking  $\text{Ca}^{++}\text{O}^{--}$  as the normal structure of CaO in the ground state it is possible to calculate the energy required to dissociate CaO into Ca and O (normal, if the lattice energy is known. Born and Gerlach<sup>7</sup> calculated the value of the lattice energy in the following manner and applied it to the Born Cycle, to get the electron affinity of oxygen. Assuming the value of the electron affinity for oxygen we can utilise the Born Cycle to find the value of R.



Here  $I_{\text{Ca}^{++}}$  is the sum of the ionisation potentials of Ca and  $\text{Ca}^+$ ;  $E_{\text{O}^{--}}$  the heat of formation of an  $\text{O}^{--}$  ion out of O and 2 electrons.  $E$  the lattice energy. Hence,

$$R + L_{\text{CaO}} = E - I_{\text{Ca}^{++}} + E_{\text{O}^{--}}$$

The value of  $E$  has been calculated by Born and

Gerlach by means of the following formula—

$$E = k \cdot \frac{n-1}{n} \cdot \sqrt[3]{\frac{\rho}{M}}$$

$$= 2450 \cdot \frac{n-1}{n} \cdot \sqrt[3]{\frac{\rho}{M}}$$

where,

$k$  = a constant involving Madelung's coefficient,  
 $n$  = the repulsion exponent,  
 $\rho$  = the density,  
 $M$  = the molecular weight.

Here  $n$  is given by the formula,

$$n = 1 + C \cdot \frac{1}{\chi} \cdot \left(\frac{M}{\rho}\right)^{1/3}$$

where  $\chi$  is the compressibility.

For CaO, SrO and BaO,  $k=2450$ , but  $\chi$  is unknown for all these compounds. Born and Gerlach have taken a rough estimate of  $75 \times 10^{-12}$  which is a mean between  $77 \times 10^{-12}$  for ZnO and  $70 \times 10^{-12}$  for MgO.

Table II gives the values of the different quantities involved in calculating  $E$  and  $E'$  which is the value of the lattice energy calculated thermochemically from

$$E' = Q + L_{Ca} + \frac{1}{2}D_{O_2} + L_{Ca^{++}} - E_O - -$$

Table II

Substance	I k. cal.	E k. cal.	M k. cal.	$\rho$	$n$	E k. cal.	E' k. cal.
CaO ...	411.7	49	56.1	3.25	5.93	800	714
SrO ...	383.0	49	103.6	4.34	9.12	758	670
BaO ...	348.2	49	153.3	5.30	11.40	728	620

It is evident that the values of lattice energy calculated from Born's equation (2) are higher than those calculated thermochemically from Born Cycle by 10 to 15 per cent. This leads to the conclusion that the assumption of approximate values of the compressibilities is wrong.

It is worth while mentioning at this place the extension made by Slater\* in the calculation of the lattice energies of alkali halides. Slater has determined the change of compressibility with temperature and extrapolated the values to the absolute zero. In some cases the values of the compressibility at absolute zero differ appreciably from those determined at room temperature, and, therefore, the energies of lattice will change a good deal. But according to Slater, the representation of the potential energy in Born's equation  $E = A + \frac{B}{r^n}$  by a single inverse term is erroneous. He, therefore, developed a series in terms of the change of compressibility with temperature and pressure, and recalculated the values of lattice energies which hardly differ from those obtained from Born's formula by 1 per cent. Since the dependence of compressibility on temperature has not been stated in a simple way, it is not possible to say whether Slater's values are better than those determined by Born's formula, as Hund has mentioned. Hence it is not possible to check the values of lattice energy unless an experimental determination is made of the compressibilities.

#### CONCLUSION

The calculations show that the behaviour of CaO, SrO and BaO in the presence of light is similar to those of the oxides and sulphides investigated

by the author. That is, the continuous absorption is due to the simultaneous transition of both the electrons in  $X^{--}$  to  $M^{++}$  so that two free and normal atoms are obtained.

*Possibility of a single electron transition.*—In the transition of a single electron from  $X^{--}$  to  $M^{++}$ , the resulting compound is  $M^+X^-$  in the process  $M^{++}X^{--} + h\nu = M^+X^-$ . The electrostatic attraction is still present. A transition  $M^{++}X^{--} \rightarrow M^+X^-$  should give bands but these were not obtained in the present work. It is quite possible that the short absorption column used in the present work was not effective in showing the bands in absorption. They might be present in the extreme infra-red or ultra-violet regions which will be investigated later on. The absence of the fundamental bands in emission of Mecke, Mahanti and others in the present absorptions shows that these bands do not correspond to transition to the fundamental level, but between two higher levels.

My sincerest thanks are due to Professor M. N. Saha, D.Sc., F.R.S., for valuable guidance and encouragement in connection with this work.

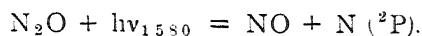
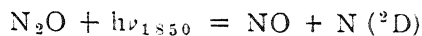
#### SUMMARY

1. The Monoxides of the Alkaline Earth Metals absorb light continuously from a long wavelength limit.
2. From thermochemical calculations according to Born Cycle it is postulated that the long wave limit of absorption corresponds to the energy required to dissociate the molecule into its constituent atoms in their normal states.

#### References

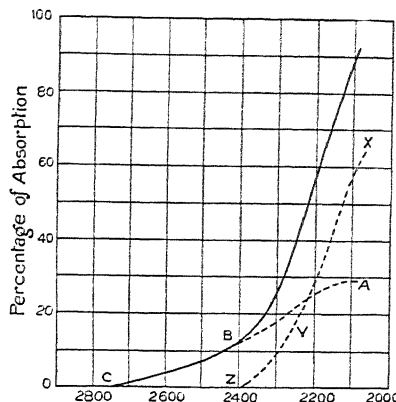
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7. Born and Gernalch, *Zeits. f. Phys.*, Vol. 5, p. 443, 1921.
8. Slater, *Phys. Rev.*, Vol. 23, p. 488, 1924.

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One of Datta's<sup>2</sup> curves for  $N_2O$  in the quartz region has been reproduced here. The point C corresponds to the long wave beginning of absorption which marks the photochemical dissociation of  $N_2O$  into NO and  $N(^4S)$ . But the curve has rather a peculiar shape. It is not smooth, but has a discontinuity at the point B. The author<sup>9</sup> has shown elsewhere that a discontinuity or Kink at any point of the absorption curve has a special significance. It has been shown that generally the main curve is not a single one but composed of two curves which have been drawn for  $N_2O$  here by dotted lines. In the figure these are ABC and XYZ which are quite distinct from each other.



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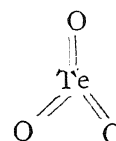
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The substances were vaporised in the vacuum graphite furnace of our laboratory<sup>11</sup> in the presence of nitrogen at atmospheric pressure. The column of vapour was nearly 15 cms. long in each case. Silica tubes were used to hold the substances. The source of light was a Hydrogen discharge tube run by a 2 KW transformer, and the copper arc was used for comparison. Photographs were taken by means of an  $E_3$  quartz spectrograph, on Schumann plates. To test the visible part of the spectrum a constant deviation spectrograph was used with either Process plates or Panchromatic plates, where necessary. Exposures were of the order of 2 to 3 minutes.

### RESULTS

In every case the spectrum was found to be continuously cut off from a long wavelength limit, and there was no trace of bands. To locate the beginning of the continuous absorption microphotograms were taken by the microphotometer belonging to the Physics Department, Muslim University, Aligarh. Here I must mention that I am very thankful to Dr. R. K. Asundi Reader in Physics there, for taking the microphotograms for me. On the same plate two exposures were given, that is, once the spot of light was allowed to run along the continuous spectrum and then the absorption spectrum.

Taking the ordinates of the continuous spectrum as 100 the percentage

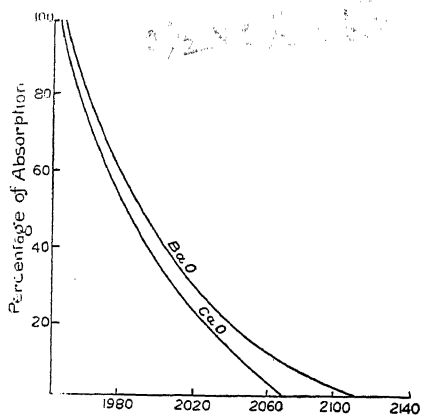


Fig. 1

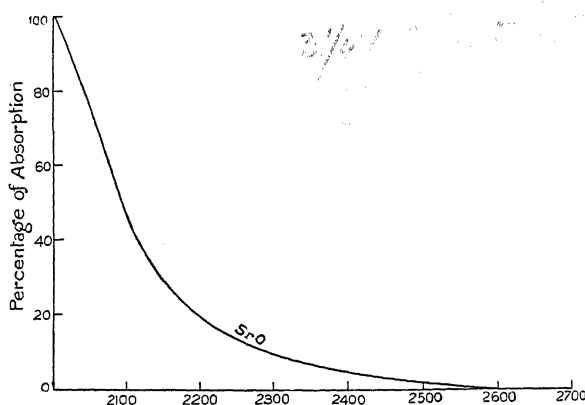


Fig. 2

of absorption was calculated from the absorption curves, and plotted with wavelength as abscissa. The point where such a curve meets the abscissa gives the beginning of absorption. From Figs. 1 and 2 we see that for CaO it is  $\lambda$  2070, for BaO  $\lambda$  2110, and for SrO  $\lambda$  2600 Å.

## CALCULATIONS

Taking  $R$  as the heat of dissociation of  $MX$  into  $M$  and  $X$  we get the following results from the Born Cycle, thermochemically.

Here  $L_{MX}$  is the Latent Heat of vaporisation of  $[MX]$ , and  $L_M$  that of  $[M]$   $D_{X_2}$  is the Heat of Dissociation of  $X_2$ .

Therefore,

$$R + L_{MX} = Q + \frac{1}{2}D_{X_2} + L_M$$

$$R = Q + \frac{1}{2}D_{X_2} + L_M - L_{MX}$$

The following table gives the values of the different quantities, the calculated values of  $R$  and the long wave limits of absorption from the graphs. The values of  $L_{MX}$  have been taken from Claassen and Veenemans' paper, and other data from Landolt and Börnstein and other tables.

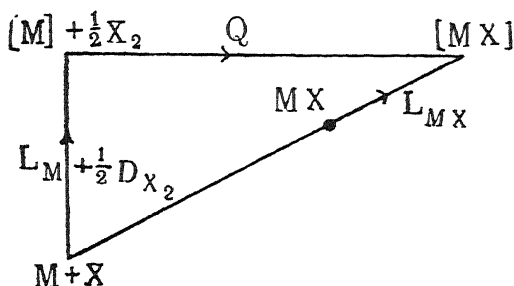


Table I

MX	$Q$ k. cal.	$\frac{1}{2}D_{X_2}$ k. cal.	$L_M$ k. cal.	$L_{MX}$ k. cal.	$R$ k. cal.	$h\nu$
CaO	145.0	64	44.2	120	133.0	$\lambda$ 2070
SrO	141.0	64	32.5	140	98.2	$\lambda$ 2600
BaO	125.9	64	32.4	90	132.3	$\lambda$ 2110

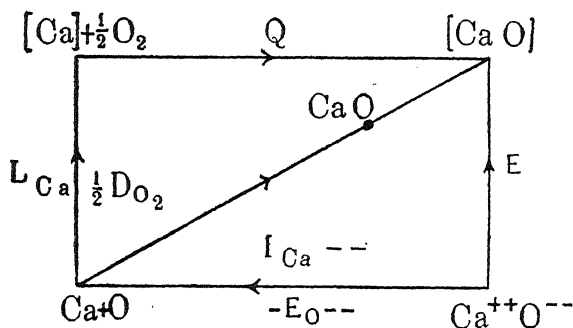
Now  $R = \frac{Nh\nu}{J}$ , and we see from Table I that there is fair agreement in the cases of CaO and BaO, but the wide divergence in the case of SrO leads one to doubt the correctness of the estimation of the latent heat of SrO. If we calculate indirectly from the long wave beginning of absorption, we find that  $L_{SrO} = 128$  k. cal.

## DISCUSSION

The appearance of the continuous absorption at the end of the spectrum in each case shows that the long wave limit of absorption is due to a transition from a firmly bound ground state to an unstable excited state. In such a case when no bands are present the binding in the ground state is supposed

to be of the ionic type. In the solid state CaO, SrO and BaO form ionic lattices of the type of NaCl. With this view in mind, therefore, we should assign such an electron structure to a compound like CaO which is also consistent with its diamagnetic behaviour. This is afforded with the structure  $\text{Ca}^{++}\text{O}^{--}$ . Here  $\text{Ca}^{++}$  and  $\text{O}^{--}$  both have the configuration  $[\ ]p^6$ , where  $[\ ]$  represents an inert gas core. The  $^1\text{S}_0$  state which results from this configuration contributes nothing to magnetism.

Taking  $\text{Ca}^{++}\text{O}^{--}$  as the normal structure of CaO in the ground state it is possible to calculate the energy required to dissociate CaO into Ca and O (normal, if the lattice energy is known. Born and Gerlach<sup>7</sup> calculated the value of the lattice energy in the following manner and applied it to the Born Cycle, to get the electron affinity of oxygen. Assuming the value of the electron affinity for oxygen we can utilise the Born Cycle to find the value of R.



Here  $I_{\text{Ca}^{++}}$  is the sum of the ionisation potentials of Ca and  $\text{Ca}^+$ ;  $E_{\text{O}^{--}}$  . . . the heat of formation of an  $\text{O}^{--}$  ion out of O and 2 electrons.  $E$  . . . the lattice energy. Hence,

$$R + L_{\text{CaO}} = E - I_{\text{Ca}^{++}} + E_{\text{O}^{--}}$$

The value of  $E$  has been calculated by Born and

Gerlach by means of the following formula—

$$E = k \cdot \frac{n-1}{n} \cdot \sqrt[3]{\frac{\rho}{M}}$$

$$= 2450 \cdot \frac{n-1}{n} \cdot \sqrt[3]{\frac{\rho}{M}}$$

where,

$k$  = a constant involving Madelung's coefficient,  
 $n$  = the repulsion exponent,  
 $\rho$  = the density,  
 $M$  = the molecular weight.

Here  $n$  is given by the formula,

$$n = 1 + C \cdot \frac{1}{\chi} \cdot \left(\frac{M}{\rho}\right)^{2/3}$$

where  $\chi$  is the compressibility.

For CaO, SrO and BaO,  $k=2450$ , but  $\chi$  is unknown for all these compounds. Born and Gerlach have taken a rough estimate of  $75 \times 10^{-12}$  which is a mean between  $77 \times 10^{-12}$  for ZnO and  $70 \times 10^{-12}$  for MgO.

Table II gives the values of the different quantities involved in calculating  $E$  and  $E'$  which is the value of the lattice energy calculated thermochemically from

$$E' = Q + L_{Ca} + \frac{1}{2}D_{O_2} + L_{Ca++} - E_O - -$$

Table II

Substance	I k. cal.	E k. cal.	M k. cal.	$\rho$	n	E k. cal.	E' k. cal.
CaO ...	411.7	49	56.1	3.25	5.93	800	714
SrO ...	383.0	49	103.6	4.34	9.12	758	670
BaO ...	348.2	49	153.3	5.30	11.40	728	620

It is evident that the values of lattice energy calculated from Born's equation (2) are higher than those calculated thermochemically from Born Cycle by 10 to 15 per cent. This leads to the conclusion that the assumption of approximate values of the compressibilities is wrong.

It is worth while mentioning at this place the extension made by Slater<sup>8</sup> in the calculation of the lattice energies of alkali halides. Slater has determined the change of compressibility with temperature and extrapolated the values to the absolute zero. In some cases the values of the compressibility at absolute zero differ appreciably from those determined at room temperature, and, therefore, the energies of lattice will change a good deal. But according to Slater, the representation of the potential energy in Born's equation  $E = A + \frac{B}{r^n}$  by a single inverse term is erroneous. He, therefore, developed a series in terms of the change of compressibility with temperature and pressure, and recalculated the values of lattice energies which hardly differ from those obtained from Born's formula by 1 per cent. Since the dependence of compressibility on temperature has not been stated in a simple way, it is not possible to say whether Slater's values are better than those determined by Born's formula, as Hund has mentioned. Hence it is not possible to check the values of lattice energy unless an experimental determination is made of the compressibilities.

#### CONCLUSION

The calculations show that the behaviour of CaO, SrO and BaO in the presence of light is similar to those of the oxides and sulphides investigated

by the author. That is, the continuous absorption is due to the simultaneous transition of both the electrons in  $X^{--}$  to  $M^{++}$  so that two free and normal atoms are obtained.

*Possibility of a single electron transition.*—In the transition of a single electron from  $X^{--}$  to  $M^{++}$ , the resulting compound is  $M^+X^-$  in the process  $M^{++}X^{--} + h\nu = M^+X^-$ . The electrostatic attraction is still present. A transition  $M^{++}X^{--} \rightarrow M^+X^-$  should give bands but these were not obtained in the present work. It is quite possible that the short absorption column used in the present work was not effective in showing the bands in absorption. They might be present in the extreme infra-red or ultra-violet regions which will be investigated later on. The absence of the fundamental bands in emission of Mecke, Mahanti and others in the present absorptions shows that these bands do not correspond to transition to the fundamental level, but between two higher levels.

My sincerest thanks are due to Professor M. N. Saha, D.Sc., F.R.S., for valuable guidance and encouragement in connection with this work.

#### SUMMARY

1. The Monoxides of the Alkaline Earth Metals absorb light continuously from a long wavelength limit.
2. From thermochemical calculations according to Born Cycle it is postulated that the long wave limit of absorption corresponds to the energy required to dissociate the molecule into its constituent atoms in their normal states.

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## CHEMICAL EXAMINATION OF THE BARK OF *NERIUM* *ODORUM*, SOLAND

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*Nerium Odorum*, Soland (N. O. Apocyanaceae) commonly known as Oleander in English, Kaner in Hindi and Karavi in Bengali and Sanskrit, is an ornamental garden plant cultivated throughout northern India. It is a well known poisonous plant of long use in Indian medicine. It has corked roots and thick and soft bark. The freshly cut bark exudes a pale yellow latex which on standing becomes resinous and sticky. It has long, slender, pointed leaves with red or white flowers. In the present investigation, the bark of the red variety was used.

As regards medicinal properties, both the root and the bark are highly useful. A decoction of the root is administered in a variety of fevers and the ground substance with water is applied in the form of a poultice on ulcers and boils. The bark is considered to be a powerful repellant and several preparations are made to be applied externally. It has been known for a long time that the active principle of *Nerium odorum* is a strong heart poison, producing powerful depression of the heart, and it is on this account that Prof. Pelikan suggested its use as a substitute for digitalis which is a well known heart depressant.

The bark of *Nerium odorum* was first examined by Greenish<sup>1</sup> and subsequently by Pieszezek<sup>2</sup> and Leulier.<sup>3</sup> They extracted from the bark a yellow aliphatic oil and a wax-like crystalline compound by petroleum ether, and two solid, bitter glucosides by alcohol. Greenish named the two glucosides as neriodorin and neriodorein. The authors did not go beyond studying the solubility of the compounds with different solvents and only the melting point of one of them is given as "above 56°". On the whole the statements of various authors regarding the active principle of the bark are very conflicting and no definite conclusion can be drawn from their work.

The present investigation was, therefore, undertaken with a view to subject the bark to a systematic chemical examination and determine its active constituents. As the result of that investigation it has now been

shown by actual isolation that the bark contains small quantities of a volatile essential oil, a yellow fixed oil, two amorphous glucosides, a solid crystalline wax, a phlobaphene, a tannin and a dark red colouring matter. The presence in the fresh bark of traces of peroxidase and a hydrolytic enzyme has also been shown.

#### EXPERIMENTAL

The dried and powdered bark was extracted with Prollius fluid and the extract tested with alkaloid reagents. A negative reaction indicated the absence of alkaloids. On ignition it left 13.5% of a white ash containing 78.0% of water soluble and 22.0% of water insoluble inorganic constituents. The soluble portion contained chlorides and sulphates of sodium and potassium together with traces of iron and the insoluble portion contained oxides of iron, aluminum and calcium together with comparatively large proportion of silica.

*Test for enzymes.*—The fresh bark on examination in the usual manner showed the presence of traces of a peroxidase (quinhydrone and purpurogallein reaction) and a hydrolytic enzyme (inversion of cane sugar). The absence of oxidase or reductase was also confirmed. In dried bark the enzymes become destroyed.

For complete analysis, 2 kilos of the dried and powdered bark were extracted with rectified spirit. The extract on cooling deposited a white flocculant precipitate which was filtered off (35 grams). The filtrate on concentration to a small volume under reduced pressure and allowing to stand deposited a sticky greenish yellow syrup from which the mother liquor was decanted off. The alcoholic mother liquor was then allowed to stand for a month pending the examination of other products.

*Examination of the white solid.*—The substance was thoroughly washed with chloroform and rectified spirit and then crystallised from boiling absolute alcohol in aggregates of colourless glistening prisms melting at 97°. The substance is sparingly soluble in cold alcohol, benzene, ether, chloroform, carbontetrachloride and petroleum ether, but dissolves to a moderate extent in hot alcohol, ethylacetate, acetone and pyridine. Concentrated sulphuric acid dissolves it with a yellow colour which slowly darkens and chars on warming. The substance was found to be a wax [Found: C=69.78, 69.84; H=11.40, 11.52%. M. W. (Cryoscopic in benzene)=461, 473.] The wax had a sp. gr. of .9804, ( $d^{30}$ ) saponification value of 53.34, iodine value of 16.2, acid value of 16.8, and unsaponifiable matter of 62.4. The acid obtained from it by hydrolysis with alcoholic potash in the usual manner crystallised from alcohol in plates melting at 93-94°. This melting point agrees with that of cocceric acid, m. p. 92-93°. The alcohol obtained from the wax after hydrolysis by extraction with ether, was recrystallised from absolute alcohol in

flattened prisms melting at 69°. From the melting point as well as other properties the substance appears to be carnaubyl alcohol, m. p. 68-70°. Hence the wax obtained from the bark of the *Nerium odorum* appears to be the carnaubyl ester of cocceric acid.

*Examination of the greenish yellow syrup.*—This was submitted to steam distillation and the distillate extracted with ether. The extract on evaporation of the ether left a small quantity of an essential oil which had the characteristic odour of the drug. The quantity obtained, however, was too insufficient for any systematic examination.

The residue after steam distillation was extracted with petroleum ether and from the extract on complete evaporation of the solvent a yellow fixed oil was obtained which had properties similar to olive oil, but this was also too insufficient in quantity for systematic examination.

*Examination of the alcoholic mother liquor.*—The alcoholic mother liquor on being allowed to stand for about a month deposited a little more of the wax which was filtered. To the filtrate water was gradually added with stirring, when a brown resinous substance was precipitated. The precipitate was filtered off (20 gms.), washed thoroughly and dried.

The dark red filtrate was treated with aqueous lead acetate which caused the immediate precipitation of a yellowish grey substance. The lead compound was filtered off, washed with water and decomposed in aqueous suspension with hydrogen sulphide. After the removal of the lead sulphide the filtrate was evaporated to dryness. The substance thus obtained was an astringent, amorphous brown powder which answered most of the properties of tannins. Thus it dissolves in alkalies with a yellow colour and in concentrated sulphuric acid with a deep red colour. Ferric chloride gives a blue-black colour and precipitate. Lead acetate gives a light yellow, gelatine solution, a colourless and tartar-emetic, a light grey precipitate. The substance shrinks at 210° and melts with decomposition at 240°. It does not reduce Fehling's solution and is very soluble in water, alcohol, acetone and pyridine, sparingly soluble in ethylacetate and completely insoluble in ether, benzene, and light petroleum. (Found: C=54.6, 54.42; H=6.78, 6.73; Pb in the lead salt=32.4, 32.8 %.)

The filtrate after the removal of the lead compound described above, gave another precipitate with basic lead acetate. This second lead salt on decomposition with hydrogen sulphide, removal of the lead sulphide and complete evaporation of the mother liquor gave a dark red amorphous powder which was found to have properties similar to tannin colouring matters. Thus it dissolved in alkalies with a dark yellow colour from which the original compound was precipitated unchanged on treatment with acid. Concentrated sulphuric acid dissolves the substance with a dark brown colour and the solution chars on heating. It does not reduce Fehling's solution. Its



general properties and solubility are allied to the substance described above. (Found: C, 53.2, 53.44; H, 5.7, 5.5%.)

*Examination of the mother liquor from the above.*—The mother liquor from the above substance was treated with hydrogen sulphide and after the removal of lead as sulphide, the filtrate was evaporated when a large amount of crystalline matter was obtained. This was found to be entirely inorganic. The mother liquor from this contained only reducing sugars.

*Examination of the brown resin described before.*—The substance was freed from oily impurities by extraction with petroleum ether and was treated in alcoholic solution with alcoholic lead acetate. The voluminous precipitate thus obtained was washed with boiling alcohol and was decomposed in alcoholic suspension with hydrogen sulphide. The filtrate on evaporation gave a dark brown amorphous powder melting at 120-122° and having a slightly astringent taste and characteristic smell of the drug. It is soluble in alkalies with a yellow colour from which it is reprecipitated with acids. It is soluble in alcohol, acetone and pyridine, sparingly soluble in acetic acid, ethyl acetate and water and insoluble in benzene, chloroform, carbontetrachloride and petroleum ether. It gives a green coloration and precipitate with alcoholic ferric chloride. From the reactions it appears to be a phlobaphene. (Found: C, 57.9, 57.53; H, 7.16, 6.73%; Pb in the lead salt 36.16, 36.3%.)

The mother liquor from the above lead acetate precipitate was treated with hydrogen sulphide to remove the excess of lead and after removal of the lead sulphide, the alcoholic filtrate was evaporated, when a bright yellow amorphous powder was obtained melting at 74° and having an extremely bitter taste. This was separated into two portions by ethyl acetate, one being soluble in the solvent and the other insoluble. The ethyl acetate soluble portion was apparently the *Neriodorin* obtained by Greenish. It is a bright yellow amorphous powder melting at 86-87° and having an extremely bitter taste. Concentrated sulphuric acid dissolves this substance with a bright red colour and nitric acid with a yellow colour. It is very soluble in alcohol, acetone, acetic acid and pyridine, moderately in ethyl acetate and insoluble in water, benzene, chloroform and ether. It does not reduce Fehling's solution except on hydrolysis. It is slightly laevo rotatory,  $(\alpha)_{\text{D}}^{30} = -1.04$ . [Found: C=65.28, 64.94; H=7.43, 7.69%; M. W.—ebullioscopic in acetone—390, 394, 388.  $\text{C}_{22}\text{H}_{32}\text{O}_7$  requires C=64.7, H=7.8%, and M. W.=408.]

The hydrolysis of *neriodorin* was carried out by moderately strong hydrochloric acid under reflux in alcoholic solution. The aglucone thus obtained was a yellow amorphous powder melting at 68°, and was altogether tasteless. The sugar obtained by hydrolysis was identified to be glucose by means of the osazone.

The ethylacetate insoluble portion of the resin described above was purified by repeated precipitation from alcoholic solution and was obtained

as a bright yellow amorphous powder melting at 106-107°. This substance has been named *Neriodorein* according to Greenish. It dissolves in concentrated sulphuric acid with a brownish violet colour and in nitric acid with an orange colour. Its general properties and solubility are practically very similar to the compound described above, *i.e.*, to neriodorin. [Found : C=56.4, 56.57; H=7.3, 7.17 %; M. W.—ebullioscopic in methyl alcohol—483, 499, 494.  $C_{23}H_{34}O_{11}$  requires C=56.79; H=7.02 % and M. W.=486.]

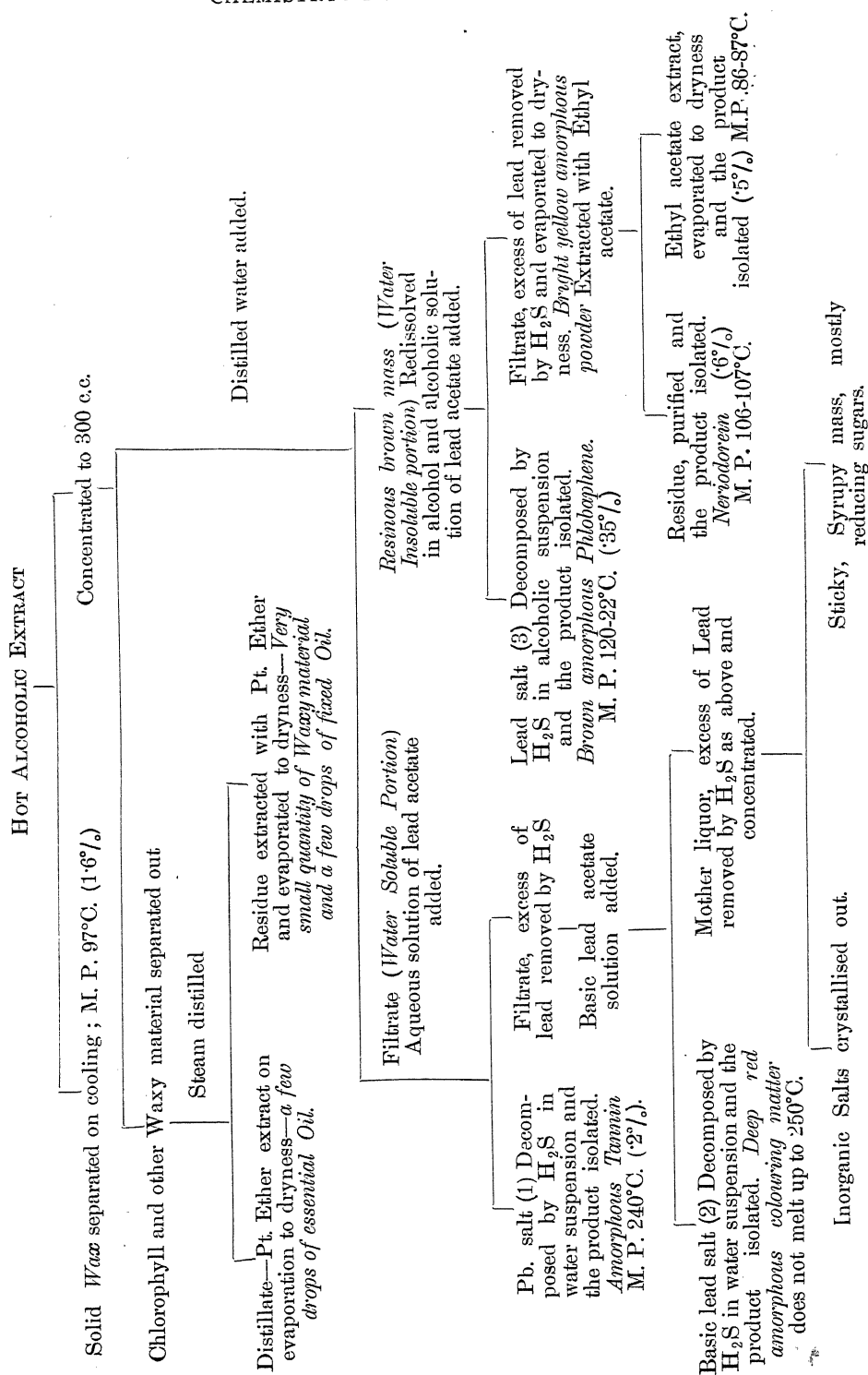
The hydrolysis of neriodorein was carried on in the same way as in the case of neriodorin. The aglucone on repeated purification from alcohol was obtained as a yellowish white amorphous powder, melting at 70°. The substance is absolutely tasteless. The sugar was identified to be glucose.

In conclusion, one of the authors (G. P. P.) desires to express his indebtedness to the "Kanta Prasad Research Trust" of the Allahabad University for a scholarship which has enabled him to take part in this investigation.

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DIAGRAMMATIC REPRESENTATION OF THE ANALYSIS OF THE ALCOHOLIC EXTRACT FROM THE  
BARK OF *NERIUM ODORUM*



# CYTOPLASMIC INCLUSIONS IN THE OOGENESIS OF PASSER DOMESTICUS

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## INTRODUCTION

Oogenesis of various kinds of animals occupies a distinctly significant place in the rank of current cytomorphic literature. The selection of bird as a type rests on its suitability as a material for investigation and the ease with which it can be obtained.

Sparrows were collected in the suburbs of Allahabad from October 1932 to February 1933 and the fixatives employed were those of Da Fano, Cajal, Ludford, Regaud, Regaud-Tupa, Zenker-Helly, F. W. A., Champy Kolatschev, Champy-Nassanov, and Bouin. Neutral red was prepared as directed by Bhattacharya in Bolles Lees' *Vade mecum*. This piece of research was carried on in the Zoological Laboratory, Allahabad University, under Prof. Bhattacharya to whom my cordial thanks are due for his constant guidance and help. The historical part has been omitted in this paper. It is exhaustively dealt with in the papers by Brambell (1925) and Das (1932).

## Observation

### *Golgi Apparatus*

In the youngest oocyte that can be obtained the Golgi apparatus appears in the form of a few discrete spherical granules seen prominently against the crystalline background of the cytoplasm (Fig. 1). At this stage the nucleus occupies the larger portion of the cell and the cytoplasm is of a uniform consistency with the few grains of the Golgi complex occupying a juxtannuclear area on the side farthest from the periphery. With the growth of the cell there occurs a definite reduction in the relative size of the nucleus, while the cytoplasm shows a corresponding increase in bulk. The Golgi bodies grow much larger in number and embrace the nucleus from all sides, thus exhibiting a decided tendency towards a perinuclear condition (Fig. 2). Ultimately the Golgi bodies completely encircle the nucleus though the greater

portion of the main mass remains confined to the original area (Fig. 3). The cytoplasm, however, fails to maintain its uniform consistency, the juxtanuclear cytoplasmic seat of the Golgi apparatus (D'Hollander's Volk Nucleus of Balbiani) being obviously of a much denser texture than the rest of the cytoplasm (Figs. 4 and 5). In the middle of this restricted area appears a clear space lodging two small granules (Fig. 4). The small dark grains scattered over the dense juxtanuclear cytoplasmic area (not shown in Fig. 4) are identified as the Golgi bodies while the clear space carrying the two granules as the centrosome lodging the diploid centrioles. This mass, later on, begins to break up in a peculiar fashion. The Golgi elements of the interior either completely disintegrate and disappear or migrate towards the periphery of the mass. The result of the process is the formation of a capsular structure with a clear central space and a sort of wall built up of Golgi bodies (Figs. 3 and 5). The clear cytoplasm of the interior is much denser and corresponds to the 'archoplasm' of other writers. This stage is of a short duration and does not always intervene as a definite step in the morphological changes the Golgi apparatus undergoes. When it does occur it is sometimes followed by a re-accumulation and further growth of the Golgi bodies. The mass ultimately breaks up and the Golgi bodies disperse through the cytoplasm till they are evenly distributed throughout. Later on, due to their outward migration, a cortical concentrated band of Golgi bodies becomes established. And this band persists even after the elements of the interior have disintegrated and become ultramicroscopic. Ultimately this band also disappears and no Golgi elements can be detected anywhere in the cell. The Golgi bodies are generally in the form of solid spherical grains and do not show the chromophilic and chromophobic parts.

#### *Mitochondria*

Like Golgi apparatus, mitochondria also occur as a few discrete granules very close to the nuclear membrane at one pole in a young oocyte (Fig. 7). With the growth of the cell they increase in number but remain confined to this pole with the result that a concentrated mitochondrial mass gets formed on this side of the nucleus (Figs. 8 and 10). The granules are scattered over a dense cytoplasmic stratum which stands out in sharp relief against the background of the nearly clear cytoplasm (Fig. 9). Obviously this is 'archoplasm' as mentioned previously. The mitochondrial mass gets later transformed into a transitory capsular structure (Fig. 10) appearing almost as a counter-part of the similarly-shaped structure mentioned in connection with the Golgi apparatus. The mass ultimately disintegrates and the elements undergo rapid dispersal till they are evenly distributed over the entire cytoplasm. This is followed by a cortical concentration of the mitochondria brought about by the outward migration of the granules of the interior. Still later three distinct concentrated

bands of mitochondria become established—one perinuclear, the second middle and the third cortical (Fig. 11). The granules in the intervening regions are few and are drifting away. The granules become very fine but could be detected even in the biggest egg the writer could examine. The mitochondria are in the form of granules and rods, the granules being the more common.

### *Egg Membranes*

The earliest oocytes are almost naked the only covering being a poorly preserved extremely thin membrane (Fig. 1). There is no sign of any follicle cell as yet and no trace of any fibrous sheath. Very shortly, however, some cells can be perceived lying irregularly around the oocyte and later, by rapid multiplication, they form one continuous covering for the egg. Between the follicular layer and the periphery of the egg there is no intervening membrane of any description. Outside the follicular layer there is a fibrous sheath of connective tissue. In the early stages this band does not show any differentiation into sharply divided layers (Fig. 13) but during the later stages of oogenesis and especially at the time when the follicular epithelium becomes many-layered a differentiation is detected in the staining capacity of the thecae. The internal layer or the theca interna stains more intensely than the theca externa. The cells of the thecae are elongated and their nuclei have a similar shape. Their cytoplasmic inclusions—Golgi bodies and mitochondria—are fine granules. The Golgi bodies of the follicular epithelium occupy a position between the nucleus and the cell wall next to the periphery of the oocyte. At this stage the epithelium is a single-layered band completely enclosing the egg and no zona radiata has yet been formed. It is at this stage that the "infiltration" of the Golgi bodies occurs (Fig. 5). The Golgi elements lying at the periphery of the cell in the figures 3 & 5 have been transported from the follicular cells and do not belong to the egg proper. The infiltrating elements are in the form of spherical granules and not solid lumps.

Figure 6 represents a much later stage. Two definite layers of fibrous sheath—theca externa and theca interna—have become established. Zona radiata, though not outstandingly prominent, is a more or less distinct structure. The Golgi bodies are uniformly scattered but the follicular epithelium is still single-layered. The Golgi apparatus of the follicle cells is a patch of fine grains situated in a juxtanuclear position, facing towards the egg proper. And these fine grains are filtering down into the oocyte. Just beneath the zona radiata lying in the periphery of the egg is a band of Golgi bodies.

The single-layered condition of the follicular epithelium persists only for a brief interval. The cells undergo rapid multiplication and with the increase in the size of the egg proper grow considerable in number. The epithelium

becomes double-layered (Fig. 11) and eventually may become multi-layered. It is generally at this stage that the zona radiata is fully formed and is distinctly perceptible. During the formation of the additional layers of the follicular cells a process of differentiation sets in. Some of the cells stain much more deeply than the others and in between the normal lightly staining cells are also found non-cellular patches which have taken a deep homogeneous stain (Fig. 8). These exhibit apparently the extreme condition to which the dark cells are reduced. The lighter cells possess the normal cytoplasmic inclusions (Golgi bodies and mitochondria). Not so the dark cells. They do not appear to possess these inclusions and have fallen into a state of decay and degeneration. The nuclei of the cells contain a number of deeply staining nucleoli. They colour deep red with acid fuchsin and intense blue with haematoxyline.

The zona radiata does not show any structure and no follicular prolongation into the substance of the zona radiata could be made out. It does not exhibit any evidence of a differentiation into two well marked layers and persists as a single, homogeneous non-striated band.

The mitochondria do not infiltrate at any stage of the oogenesis, and are present in the follicle cells as fine grains. Like Golgi bodies they occupy the juxtannuclear position facing the egg periphery.

In close proximity to the thecae occur numerous luteal cells presenting a glandular appearance.

The follicle cells sometimes show abnormal activity. They multiply rapidly and invade the cytoplasm of the egg and eat away, as it were, the entire egg.

#### *Yolk-bodies*

An examination of the osmic-treated material clearly shows that only one kind of yolk is present—the fatty yolk. There is absolutely no trace of any albuminous yolk. In osmic-fixed preparation these bodies appear dense black and not pale-yellowish as is often, though not always, the case with albuminous yolk, and a short immersion in acid-free turpentine decolourizes them. They are never fixed by the current non-osmic fixatives and are extremely unstable. Even the xylol of Canada balsam dissolves them out in the mounted sections (Fig. 5). Staining with Sudan III gave the confirmatory reaction of fat and they got coloured with neutral red if left in that vital dye for a sufficiently long period, say 90 to 100 minutes. They never stain with acid fuchsin.

The centrifuge experiment throws them to the upper pole, these being the lightest inclusions. The centrifuge experiment also clearly demonstrates the absence of albuminous yolk. The empty vacuoles arranged on the cortex in non-osmic preparations are fatty yolk vacuoles which were not

preserved by the fixatives and were washed out in the subsequent process (Fig. 6).

The fatty yolk bodies arise in the juxtanuclear area and also on the cortex. It is usual to find them arranged on the periphery but in many cases they fill the interior as well.

The fatty yolk does not arise independently in the ground cytoplasm but in intimate association with the Golgi bodies which are mainly responsible for their formation. All gradations between the minute particles of the Golgi bodies and the swollen spheres of yolk are present (Fig. 12). The periphery is crowded with big and small spheres of fatty yolk with the Golgi bodies scattered in between them. Besides, on treating the sections with acid-free turpentine or immersing the slide for a long time in xylol yolk dissolves out but black crescents and granules are left bordering the empty vacuoles. (Fig. 13).

With the growth of the oocyte the fatty yolk bodies increase enormously in number and also swell up to attain greater dimensions. There is, however, distinctly perceptible a stage beyond which the fatty yolk bodies tend to become distinctly smaller though concomitantly more numerous.

An abundance of fatty material is found to be filling very young oocytes in certain cases. This fat is much more unstable than the ordinary Golgi yolk and in fixed preparations the cells containing them present a highly vacuolated appearance. The archoplasm stands out in strong contrast in such cells. This appears to be a case of fatty degeneration and not a normal process.

### *Intravital*

Examination of thin strands of ovary in salt solution under strong artificial light (1000 candle power) did not show the Golgi bodies. The younger cells were comparatively free from yolk globules and the cytoplasm appeared a clear crystalline expanse containing a few refringent granules. In very young oocytes a dense juxtanuclear area corresponding to the "Yolk Nucleus of Balbiani" could be picked out. The older cells were densely packed with fatty yolk spheres.

Treatment of fine pieces of ovary with neutral red brought out Parat's vacuome. In early cells these red granules were confined to the "Yolk Nucleus of Balbiani" area but in more advanced ones they were scattered throughout the cytoplasm (Fig. 14). These minute red granules were generally scattered individually but were also found in groups (Fig. 14). Occasionally some of them ran into each other to form a single big structure



(Fig. 14). The "Vacuome" of the follicle cells are likewise in the form of fine red granules scattered throughout the follicle cells. It takes nearly an hour before the vacuome are distinctly seen, though half an hour may suffice in some cases. After the vacuome have clearly come out a little quantity of 1% osmic acid is run into from one side and drained from the other. The Golgi bodies come out as homogeneous grains and crescents. In the advanced oocytes they are homogeneously distributed (Fig. 14) but in young eggs they are confined to the juxtannuclear area. The Golgi bodies of the follicular epithelial cells are minute particles mostly gathered in a patch near the nucleus.

A simultaneous application of neutral red and janus green B met a failure. Pieces of ovary separately treated with janus green B showed mitochondrial patches with remarkable clearness. Mitochondria were in the form of rods and granules situated in a patch on a denser substratum of cytoplasm. These patches were regularly arranged concentrically round the nucleus (Fig. 15) and the arrangement continued in sufficiently advanced oocytes.

The mitochondria of the follicular cells were invariably found in the form of a prominent juxtannuclear patch facing the periphery of the egg.

Fatty yolk spherules get blackened on prolonged treatment of the tissue with osmic acid. But for the confirmation of the presence of fat Sudan III test is more satisfactory. It was used on fresh and formalin-treated material and in both cases showed the usual red reaction.

## Discussion

### *Golgi Apparatus*

By a majority of prominent cytologists the Golgi body is treated as a permanent structure independent of any canalicular or vacuolar apparatus and possessing a fundamental chemical and structural basis in spite of the diversity of form and function it may assume in different varieties of cells. It is credited with the power of growth, assimilation, and fission and is considered a cytoplasmic inclusion of universal occurrence. It is not considered a product of metabolism.

Still there is no undisturbed unanimity. Walker and Allen (1924) openly condemned Golgi apparatus as an artefact produced by the action of fixatives on the cytoplasmic colloids. Benseley and Helen (1929) showed that osmication involves no chemical reaction but is simply a process of reduction, dispersal and adsorption. Strangeways and Canti (1927) did not find Golgi bodies in their dark ground experiment on tissue culture cells. Chambers (1931) in the course of his micro-dissection found no area of resistance in the cell.

The Golgi apparatus has, however, been seen intravital. Gatenby easily observed them in male germ cells of *Cavia* and *Abraxas*, by applying neutral red. Gatenby, Rau, and Brambell were able to produce microphotographs of the Golgi bodies of living male germ cells. Bhattacharya and Das (1929) demonstrated it by vital methods in the oocytes of pigeon. Nath saw the apparatus intravital in the eggs of earthworm and frog. Das (1932) observed it in the living unstained oocytes of pigeon.

The observation of the present writer is in conformity with those of the latter group. The Golgi apparatus is easily seen on treating the fresh ovary with 1% osmic acid for a short time and though the method is not strictly intravital, it does not involve any danger of artefact. (Strangeways and Canti 1927.)

The morphology of the Golgi apparatus admits of extensive variation. Cajal considered it a system of canals with a limiting membrane enclosing lipoids. Hirschler (1913, 1914, 1916, 1918) advanced the theory that the Golgi apparatus is of a duplex character and essentially lamellar in construction. An existent representative of Hirschler "Apparatinhalt" was found by Bowen in the chromophobe of the apparatus. Kunze 1921, Brambell 1923, Brambell and Gatenby 1923 showed that in the nerve cells of *Helix* the Golgi body consists of curved rods or ring-shaped dictyosome. Harvey showed it to be scaly in form but Nath in a series of papers on oogenesis declared that the only form of the apparatus is vesicular with chromophilic cortex and chromophobic interiors. Bhattacharya described many forms in the tortoise,—rodlets, platelets, crescents, beaded strings, etc. (1925).

In the material under investigation the Golgi bodies were mostly in the forms of spherules, rods and crescents. It does not, however, seem necessary to reduce the morphology of the Golgi apparatus to a standard form.

The functional significance of the apparatus in oocyte remains obscure. In many cases it has been shown to form fatty yolk. But beyond this little more is known. In male germ cells it forms the "acrosomes" (Bowen, Gatenby and others) and in glands it is held responsible for the formation of the secretory material. Its function thus varies with the nature of the cell in which it is lodged. In female germ cells its only function known so far is to supply nutriment to the developing ovum. In the eggs of *Passer domesticus* the Golgi apparatus is concerned with the same function.

### *Mitochondria*

Mitochondria have been seen intravital in the eggs of a number of animals by workers in this laboratory. The dark ground experiments of tissue culture conducted by Strangeways and Canti (1927) conclusively showed their presence in living tissues and Chamber's report (1931) is equally

confirmatory. In the present work mitochondria were seen by intravital methods very clearly.

In the eggs mitochondria are generally present in the form of granules and rods. In a few cases the filamentous forms have also been described; (Hibbard in *Discoglossus*, Hibbard and Parat in *Pygostens* and *Perca*, Harvey in *Lumbricus*, King in *Peripatus*, Bulliard in *Emy lutaria*, Gatenby in *Apanteles* and Bhattacharya and collaborators in a number of animals from our laboratory).

The present writer finds only granular and rod-like mitochondria in the eggs of *Passer domesticus*.

Very early oocytes of the material under investigation were entirely free from mitochondria. Gardiner in *Limulus* and Harvey in *Carcinus* met a similar failure and a number of workers from this laboratory have reported similar cases. The continuation of mitochondria from the undifferentiated germ cells to the segmenting ovum is, however, a well-established belief among the cytologists and there is a wealth of evidence to support it. It is possible that at very early stages they are in ultra-microscopic condition and hence escape detection.

Mitochondria play absolutely no part in the process of vitellogenesis of the oocytes of *Passer*. This is in sharp disagreement with the reports of Brambell on the oogenesis of domestic fowl and of Das on pigeon who ascribe the formation of proteid yolk to mitochondria.

Van Durme's account of mitochondria contains a description of three zones into which the mitochondria get concentrated during the early stages of dispersal. These three zones are prominently seen in the eggs of sparrow but they never give rise to proteid yolk. Brambell does not record these layers and Das mentions only an ultimate cortical concentration. This cortical band is an intermediate stage in the oogenesis of *Passer domesticus*.

#### *Egg-Membranes*

The mature egg of this bird is enveloped by theca externa, theca interna, follicular epithelium and zona radiata. I have not met the cortical fibrillated layer situated between the periphery of the cell and the zona pellucida as mentioned severally by Gatenby and Bhattacharya. And the zona radiata is a single and not a permanently double-layered structure. Das (1932) thinks this fibrillated layer to be the vacuolated part of the cortex. There is no such vacuolated area in the cortex of the oocytes of the *Passer*, the only vacuoles being those of the fatty yolk. What Gatenby and Bhattacharya described as fibrillated layer is probably a separate structure not found in birds.

The zona radiata has been described as marked by definite radiations and traversed by hollow strands of cytoplasmic material of the follicle cell in fishes, reptiles, mammals and birds (Loyez, Van-der Stricht, Champy, Thing,

etc.). The zona radiata of Passer is unstriated and does not show any prolongation of follicular substance.

Hall, Brambell, Mertens and Das described two kinds of cells in the follicular epithelium of the oocytes of birds and fishes—clear and dark cells. My observation is in agreement with those of these workers. The differentiation of the dark cells from the light normal cells is brought about by a process essentially degenerative in character, the non-cellular dark patches being the extreme stage. These dark patches seldom or never extend across the full breadth of the follicular layer and there is little reason to ascribe a mechanical function to these, as Brambell has done. Further, it is significant to note that nearly all the follicular cells in cases of abnormal activity are like dark cells—dark and devoid of the usual cellular inclusions.

The nutrition of the oocyte demands an inflow of nourishing substance through the enveloping layers and the actual occurrence of such a process has been known for long (Loyez 1905, Waldayer 1870). The nature of granules filtering down into the cytoplasm of the egg has been only recently investigated (Bhattacharya and Brambell). The infiltration in the oogenesis of this bird occurs both before and after the formation of the zona radiata. Das (1932) came to a similar conclusion, but Brambell has recorded only one stage. The significance of the intrusion of the Golgi bodies becomes apparent on the assumption of their nutritive function.

#### *Vitellogenesis*

For a bird the absence of albuminous yolk is rather an exceptional fact. Van Durme (1914), Brambell (1925) and Das (1932) all found it and traced its formation to the mitochondria. Mitochondria have been held responsible for albuminous yolk formation by various other workers in different animals (Hirschler, Bhattacharya, Nath, Lams and Doorme, and Bhattacharya and collaborators from this laboratory). Nucleolar extrusions have been ascribed the same function in many cases (Gatenby, Nath, King, Gresson and others).

Weiner, Harvey and Steopoe derived albuminous yolk from Golgi bodies, while Nath, Parat and Hibbard and Parat declared "vacuome" to be the source in some cases. It is difficult to accept the former interpretation as of late the Golgi apparatus is being considered the main source of fatty yolk formation (Gatenby, Ludford, Brambell, Nath, King, Bhattacharya, Das and others). The observation of the present writer is in agreement with the interpretations of the latter group.

#### *Vacuome*

Parat's vacuome hypothesis considers the classical Golgi apparatus an artefact created by metallic precipitation in and around a system of neutral red-stainable vacuoles which are the real pre-existent organelles of the cell.

Benseley first described a canalicular apparatus in the cell and Guilliermond and Mangelot later emphasized the canalicular nature of the Golgi apparatus in plant cells. Guilliermond extended the hypothesis to animal cells. But a complete vacuolar hypothesis was put forth by Parat and Painleve (1924).

Parat contended that under the influence of cytological fixatives the vacuoles get disorganised, run and coalesce together to form the typical Golgi network. His work centred mainly round secretory materials. Krjukowa (1929), Beams and Goldsmith (1930) repeated Parat's work on salivary gland cells of chironomus larva and reported that they did find the typical crescentic Golgi bodies which Parat had overlooked.

Parat, however, had to explain the dictyosomes stuck over the archoplasm in many germ cells (Gatenby, Bowen, etc.) Parat called them "*chondriome actif*" or modified mitochondria while the vacuoles they enclosed were termed the "vacuome." Avel, Gatenby, Bowen, Pollister and others definitely rejected this view. This "Lepidosome" theory never attracted any enthusiastic support.

Parat's vacuome theory was supported by workers on protozoan Cytology (Joyet and Lavergne, Hall, Hirschler, Volkonsky, Lwoff and Lwoff, Cowdry and Scott and many others). Covell and Scott as a result of their neutral red experiments on spinal Ganglion cells came to the same conclusion. Beams (1931) emphatically contradicted this and showed that the interpretation of Covell and Scott was erroneous.

Neutral red itself does not seem to be a very specific dye. Vera Koehring (1929) coloured several system of Protozoa with it which cannot be homologized with Parat's vacuome. Then the work of Chlopin (1927, 1928), Weiner ('30) shows that the neutral red, though the least toxic vital stain, is responsible for creating artefacts and thus causes misinterpretation. In his latest paper on chironomus larva salivary gland cells Gatenby showed that neutral red created artificial spaces that were mistaken for pre-existent structures (Vacuome) and that the independently occurring Golgi bodies were real but separate bodies.

Moreover, the Golgi apparatus and vacuome have been seen simultaneously by many workers (Tretjakoff, Grabowski, Rumjantzew, Beams, Beams and King, Bhattacharya and Das, and Nath). The separate roll of the two structures in spermatogenesis was worked out by Voinova, Payne, Pollister, Hirschler, Monne and Gatenby.

In the oocytes of *Passer domesticus* the two structures are completely independent and have been simultaneously demonstrated. There is no reason to suppose the vacuome to be a secretory product of the Golgi body and at the same time it is difficult to treat "Vacuome" as a mere artefact. They cannot be brought out in dead cells and did not collapse on subsequent osmication.

*Nucleolar Extrusion*

There is no sign of nucleolar extrusion in this animal.

## SUMMARY

In the oogenesis of *Passer domesticus*

1. The Golgi bodies appear in the form of a few granules in young oocytes, grow in number, form the "Yolk-Nucleus of Balbiani" with a clear central space, spread out, and get arranged on the cortex in a concentrated band. They ultimately disappear.
2. Centrosomes and centrioles have been demonstrated in the Yolk Nucleus of Balbiani area.
3. Golgi bodies give rise to fatty yolk.
4. Mitochondria appear in early oocytes as a few granules which increase in number, form Yolk Nucleus of Balbiani, then spread out and form three concentrated layers.
5. There is no albuminous yolk.
6. Vacuome and Golgi bodies have been shown to be independent structures and are shown intravital.
7. Mitochondria have been demonstrated by intravital methods.
8. There is no sign of nucleolar extrusions.

## EXPLANATION OF LETTERING

G. B.	...	Golgi Bodies.	Mi. P.	...	Mitochondrial Patch.
Mi.	...	Mitochondria.	Va.	...	Vacuome.
Y.N.B.	...	Yolk Nucleus of Balbiani.	G. Gr.	...	Golgi granules.
F.G.B.	...	follicular Golgi bodies.	G. Cr.	...	Golgi crescents.
F.C.	...	Follicle Cells.	F.Y.B.	...	Fatty Yolk body.
F.N.	...	Follicular Nucleus.	C.S.	...	Central space.
F. Nu.	...	Follicular Nucleolus.			
N.	...	(Oocyte) Nucleus.	B.V.	...	Blood Vessel.
L.M.C.	...	Layer of Mitochondrial Concentration.	F.V.	...	Fatty vacuoles.
Inf.	...	Infiltration.	V.	...	vacuoles.
Th. in.	...	theca interna.	L.C.	...	Light Cell.
Th. ex.	...	theca externa.	D.C.	...	Dark Cell.
Z.R.	...	Zona Radiata.	N.C.P.	...	Non-cellular Patch.

## EXPLANATION OF PLATES

Fig. 1. Early oocyte showing a few juxtanuclear Golgi granules. Da Fano, toned.

Fig. 2. More advanced oocyte. Golgi bodies have grown in number and are spreading on sides Da Fano, toned.

Fig. 3. Golgi bodies have surrounded the nucleus. Yolk Nucleus of Balbiani with a clear central space. Infiltrated Golgi bodies are on the periphery. Da Fano toned.

Fig. 4. Yolk Nucleus of Balbiani with centrosomes lodging centrioles. Da Fano toned and stained with Iron-alum haematoxyline.

Fig. 5. Yolk Nucleus of Balbiani with the central space. Golgi bodies are spreading out and fatty vacuoles are present. Infiltrated Golgi bodies are on the periphery. Ludford bleached.

Fig. 6. Infiltration of Golgi bodies. Zona radiata is forming and thecae are two-layered (Cajal).

Fig. 7. Young oocyte showing a few mitochondrial granules. F.W.A. Acid Fuchsin.

Fig. 8. Yolk Nucleus of Balbiani. F. W. A. Acid Fuchsin.

Fig. 9. More advanced oocyte. Mitochondria are present in two patches and are spreading out. F.W.A. Acid Fuchsin.

Fig. 10. Microphotograph of an advanced oocyte showing a juxtannuclear patch of mitochondria with the central space. F W. A. Iron-alum Haematoxyline.

Fig. 11. Mature oocyte. Mitochondria are arranged in three concentrated layers. Zona radiata is formed; dark cells and non-cellular patches are formed. Follicular epithelium is two-layered. Thecae have differentiated into two layers.

Fig. 12. Part of an old oocyte showing the formation of fatty yolk bodies. Theca externa, theca interna, and blood vessels are shown. (Ludford.)

Fig. 13. Showing Golgi bodies left after dissolving out fat. (Ludford.)

Fig. 14. Part of an old oocyte showing vacuomes and Golgi bodies. Neutral red and Osmic.

Fig. 15. A young oocyte showing concentric mitochondrial patches. Janus Green B.

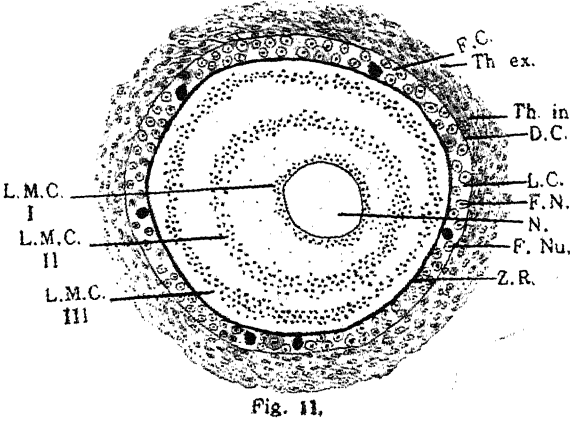
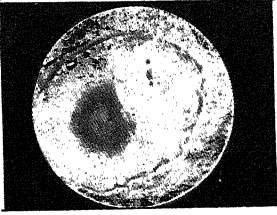
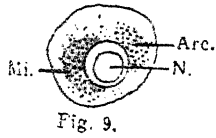
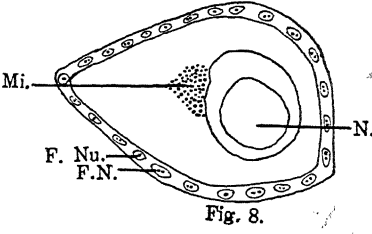
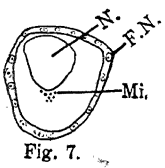
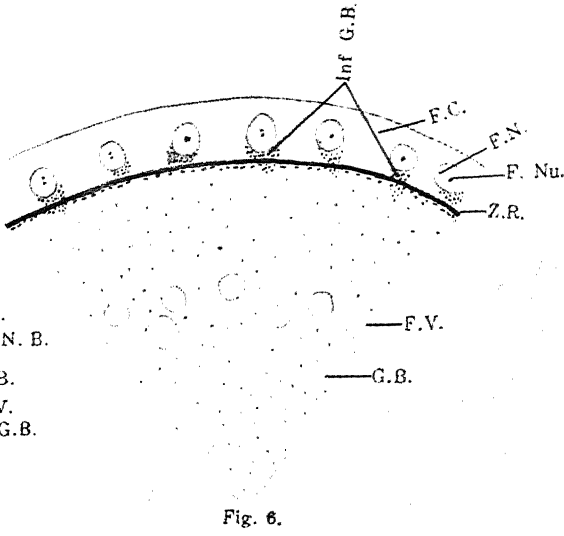
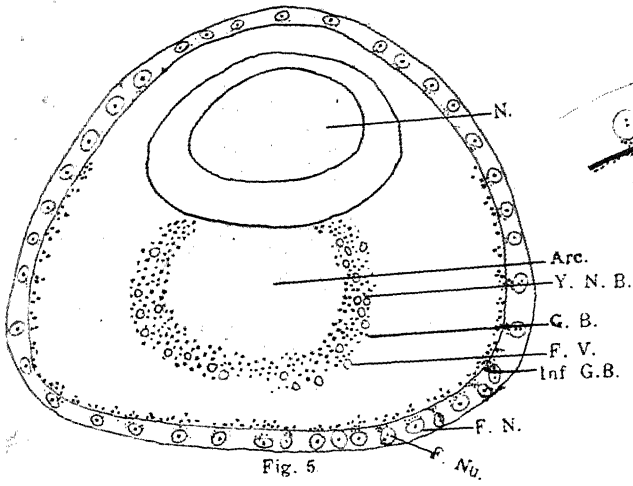
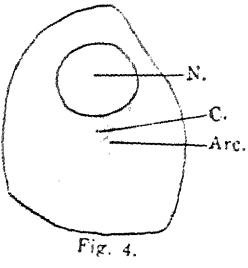
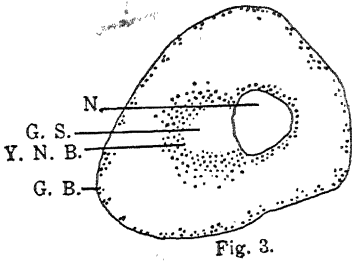
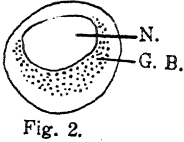
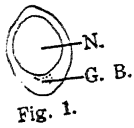
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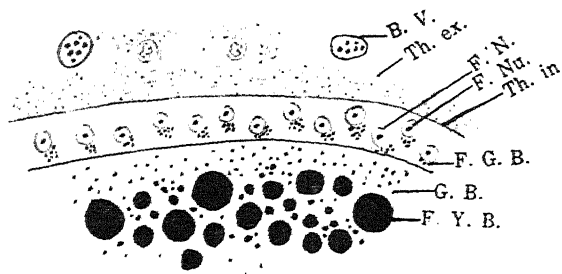


Fig. 12.

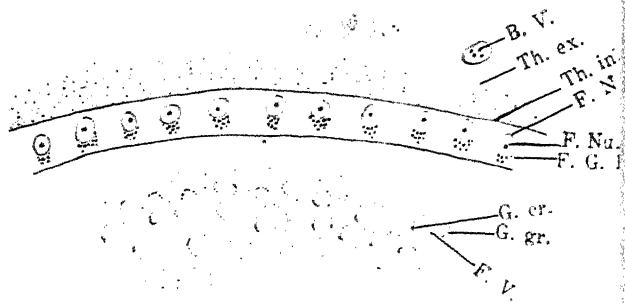


Fig. 13.

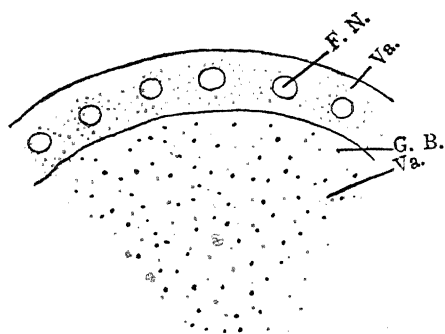


Fig. 14.

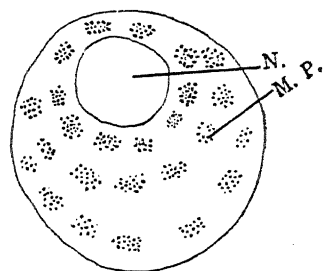


Fig. 15.

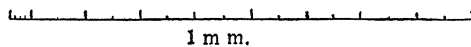


Fig. 1,2,3,4,5,6,7,8,12,13,14,15.



Fig. 9,11.



# ON THE ABSORPTION SPECTRA OF THE HALIDES OF ELEMENTS OF THE FIFTH GROUP

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Communicated by Prof. M. N. Saha

Received October 5, 1933

This paper is an extension of my previous work<sup>1</sup> on the absorption spectra of some of the lower chlorides of the elements of this group. The substance investigated are the bromides and iodides. I have also examined antimony penta-chloride to see if there was any correspondence between the results obtained in the case of antimony trichloride previously examined and those obtained here.

As was said in the previous paper<sup>1</sup> these salts can be regarded as saturated compounds. As an illustration we may consider  $\text{PI}_3$ . Its constitution may be given by  $\text{P}^{+3} 3\text{I}^-$ . Each  $\text{I}^-$  —ion is diamagnetic and  $\text{P}^{+3}$  has the constitution  $1s^2 2s^2 2p^6 3s^2$ ; hence  $\text{P}^{+3}$  is also diamagnetic. The case of bromide is similar and the same is the case with the halides of other elements of this group. All these substances should show continuous absorption.

Their atomic heats of formation are calculated as in the previous paper. They are calculated by the help of the following formula which is based on the Born cycle. In the case trihalides the atomic heat of formation in kcals is

$$R = Q + L_M + \frac{3}{2} D_{\text{Ha}_2} - L_{\text{MHa}_3}$$

where  $Q$  = heat of formation of the salt in kcals per mole.

$L_M$  = heat of vaporisation of the metal from the condensed state to the atomic state in kcals per mole.

$D_{Ha_2}$  = heat of dissociation of the halogen (Ha) in kcals per mole.

$L_{MHa_3}$  = heat of vaporisation of the salt in kcals per mole.

$L_M$  is in its turn calculated by the help of relation

$$L_M = \frac{L + D_1 + 2D_2}{4}$$

where  $L$  = heat of vaporisation of the metal from the condensed state to a vapour of tetra-atomic molecules.

$D_1$  = heat of dissociation of a tetra-atomic molecule into diatomic ones in kcals per mole.

$D_2$  = heat of dissociation of the diatomic molecule into monatomic ones.

As an example we take the case of  $AsI_3$ . Here  $Q = 28.8$  kcals;  $S = 26.5$  kcals;  $\frac{3}{2} D_{I_2} = 53.4$  kcals and  $L_{AsI_3} = 19.2$  kcals; whence we get the value for  $R$  to be equal to 89.5 kcals.

All the necessary data have been taken from Landolt and Bornstein's tables and from Mellor's Treatise on Inorganic and Physical Chemistry. In the case of certain compounds, however, the value for latent heat of vaporisation of the compounds is not given as such. In that case we obtain this value from the data for vapour pressures of the substance at various temperatures. The formula employed for getting this result is

$$L \text{ in cal.} = \frac{T_1 T_2}{T_2 - T_1} \log_e P_2 / P_1$$

where the pressures  $P_1$  and  $P_2$  correspond to temperatures  $T_1$  and  $T_2$  respectively given in absolute scale.

#### EXPERIMENTAL PROCEDURE AND RESULTS

The experimental procedure was similar to that described in my previous paper.<sup>1</sup> In certain cases the absorption began in the quartz region, whilst in others it began in the glass region. In the former case the source of radiation was a hydrogen tube run by a transformer with a current density of 100 mA. The spectrograph was a quartz one of  $E_3$  type. The plates used were process plates. In the other case the source of radiation was a pointolite lamp, the spectrograph was a glass one of the constant deviation type and the plates were Agfa panchromatic.

In the case of antimony pentachloride the substance was a liquid and it was kept in a side bulb which was maintained at the temperature of boiling

water. According to tables the saturated vapour pressure at this temperature is 63 mms of mercury, while the pressure recorded in the manometer attached to the tube is 60 mms. The absorption tube was then heated successively to the temperatures mentioned in the horizontal row of table 2. Microphotographs of these plates were taken at the laboratory of the Muslim University, Aligarh, by Dr. Asundi and the beginning of the long wavelength limit of absorption could be easily inferred from these records. It was found that at the lower temperatures, the rise of the absorption curve was gradual and became more so when the temperature was increased. The long wavelength limit was found to be continuously shifting from the value  $\lambda$  3500 Å at 100°C. to  $\lambda$  4100 Å at 340°C. The experiment, therefore, shows that the long wavelength limit shifts with the temperature. To see if there was any retransmission, the furnace was kept at room temperature and the pressure of the vapour in the absorbing column was varied from 1 mm. to 76 cms. of mercury by gradual steps. It was found that there was no retransmission.

The other substances were solids. For the sake of convenience, a little of solid was placed in each case inside the absorption vessel and heated to the temperatures mentioned in the top row of table No. 2. Both the pressure and the temperature of the absorbing column were thereby varied. The long wavelength limit was determined in the same way as before. Generally at lower temperatures, the absorption curve was found to be gradual and it became sharp as the temperature was raised. For  $\text{SbBr}_3$  the effect was more marked.

All these substances were examined to see if there were retransmission in any of them. For that purpose the temperatures to which they were heated was varied from the room temperature to a maximum of 340°C. by very gradual steps. This ensured that the pressure of the absorbing column was changed from very low values to higher ones very gradually.

In all these cases with the exception of  $\text{AsI}_3$  and  $\text{SbBr}_3$ , no other change except the shift in the long wavelength limit of absorption could be noticed with the changes in the conditions of temperature and pressure. The long wavelength shift was, in each case, towards the red end of the spectrum.

In the case of  $\text{AsI}_3$  and  $\text{SbBr}_3$  there was, in each of them, a retransmission of light following a continuous absorption which in its turn terminated in another continuous absorption. Thus there were two long wavelength absorption limits in these two cases corresponding to the two continuous absorptions separated from one another by the small patch of retransmission. The retransmissions appear at a comparatively high temperature, which shows that a comparatively large pressure of the absorbing column is required to make those retransmissions appear. These results are given in table 3. It was found that as the temperature increased the retransmitted patch



contracted from both sides. A similar phenomenon was observed in the case of tinhalides.<sup>4</sup>

The microphotograms of the various absorption spectra are given in figures 1 to 6. For economy of space all the microphotograms for one substance taken under various conditions are compressed in one figure. The microphotograms have been taken along the spectra. The microphotogram is (A) of the continuous spectrum from the hydrogen tube, (B) of the continuous spectrum from the pointolite lamp, (1) the absorption spectrum of the vapour of the substance at 50°C., (2) the absorption spectrum at 100°C., (3) the absorption spectrum at 300°C., and (6) the absorption spectrum at 340°C.

Table 1

Substance	Long wavelength limit in $\text{\AA}$ " $v_m$ "	$Q_m = \frac{Nh\nu_m}{J}$ in kcal	Heat of reaction required to convert solid element into monatomic vapour in kcal per gm. atom " $L_m$ "	Heat of formation of the salt in kcal per mole " $Q$ "	Heat of dissociation of the halogen molecule into atoms in kcal per mole " $D$ "	Heat of vaporisation of the salt in kcal per mole " $L$ "	$\frac{R}{3}$ in kcal for trihalides and $\frac{R}{5}$ in kcal for pentahalides	$Q_m - \left[ \frac{R}{3} \text{ or } \frac{R}{5} \right]$ in kcal
Antimony pentachloride	4130	68.9	[27.6]	104.8	56.87	11.05	52.7	16.2
Arsenic tribromide	3248	87.6	26.5	59.1	45.22	[10]	[47]	47.6
Antimony tribromide	5450	52.2	[27.6]	76.9	45.22	3.5	56.2	-4.0
Phosphorus triiodide	3147	87.4	34.5	26.7	35.6	[12]	[34]	53.4
Arsenic triiodide	5616	50.6	26.5	28.8	35.6	19.2	29.8	20.8

N. B.—1. The values enclosed in brackets are uncertain because of the interpolation referred to in my previous paper.

2.  $v_m$  corresponds to that long wavelength limit of absorption which is nearest to the red in those cases where there are more than one absorption limits.

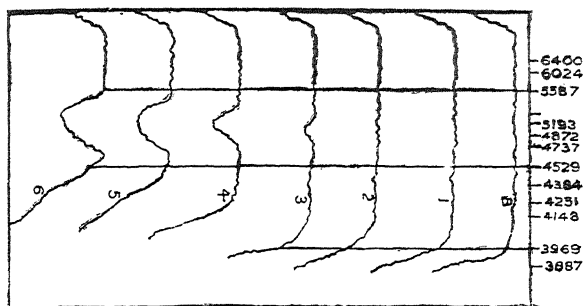


Fig. 1

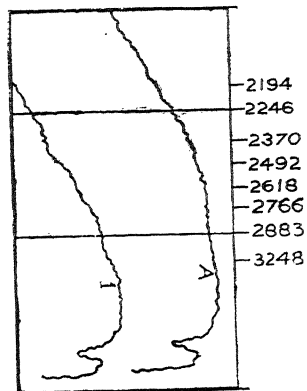


Fig. 2

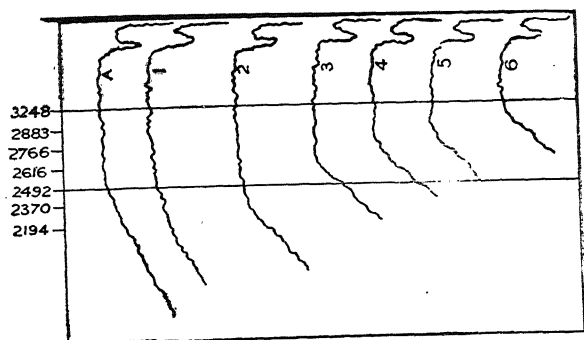


Fig. 3

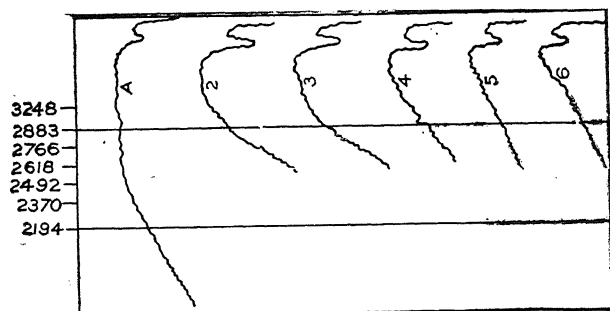


Fig. 4

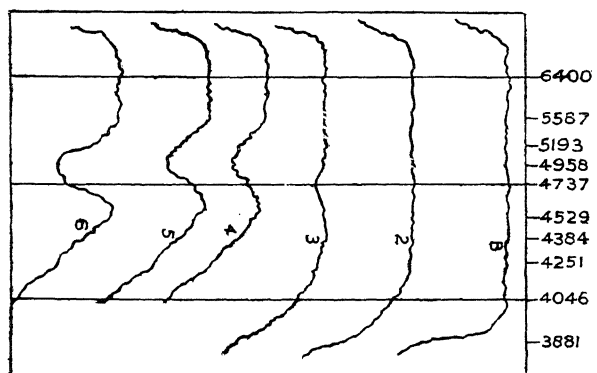


Fig. 5

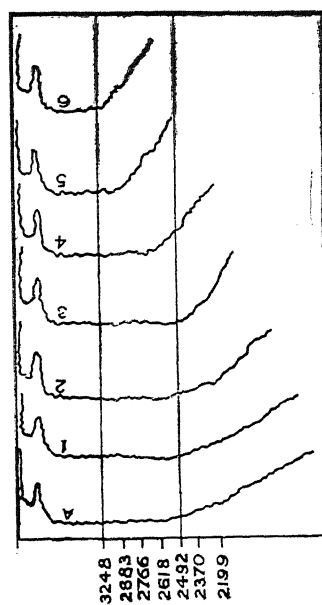


Fig. 6



Table 2

*Long wavelength limit of absorption of the vapours of the various substances at different temperatures in Angstrom units.*

Substance	Temperature in °C					
	50	100	180	250	300	340
Antimony pentachloride	...	3500	3670	3820	3965	4100
Arsenic tribromide ...	2100	2390	2595	2815	3020	3240
Antimony tribromide ..	3200	4110	4920	5444	5443	5450
Phosphorus triiodide ...	2095	2280	2485	2700	2920	3120
Arsenic triiodide ...	4002	4100	5070	5300	5465	5600

Table 3

Substance	First long wavelength limit of absorption "ν <sub>m<sub>1</sub></sub> "		Second long wavelength limit of absorption "ν <sub>m<sub>2</sub></sub> "		ν <sub>m<sub>2</sub></sub> - ν <sub>m<sub>1</sub></sub> cms. - <sup>1</sup>	Difference between the energies of the first two metastable states of the corresponding halogen (2P <sub>1/2</sub> - 2P <sub>3/2</sub> ) cms. - <sup>1</sup> .
	Å	cms. - <sup>1</sup>	Å	cms. - <sup>1</sup>		
Antimony tribromide	5260	19011	4670	21413	2402	7600
Arsenic triiodide	5440	18349	4600	21739	3390	3700

## DISCUSSION

Saha and Datta's hypothesis<sup>2</sup> says that the long wavelength limit of the absorption spectrum of the vapour of a substance which can be represented by MX<sub>n</sub> is given by  $\frac{R}{n}$  where R is its atomic heat of formation. We find, however, that the energy of optical dissociation is in each case different from one-third of the thermochemical value in the case of trihalides and one-fifth for pentahalides. As found in the chlorides<sup>1</sup> of this group the value is in excess to the thermochemical one. But this excess is not general. In the case of

antimony tribromide, for instance, the energy of optical dissociation is 52.2 kcal, whereas one-third of the thermochemical energy is 56.2 kcal. The thermochemical value is, however, not quite reliable. Its calculation involved the use of the value of the heat of vaporisation of antimony from the condensed state into monatomic vapour. This was obtained only by interpolation, on which much reliance cannot be placed. This deviation from the general behaviour may, therefore, be only fortuitous.

In the cases of  $\text{AsBr}_3$  and  $\text{PI}_3$  the atomic heats of formation ( $R$ ) are  $(153.4 - L_{\text{AsBr}_3})$  and  $(114.6 - L_{\text{PI}_3})$  kcal respectively. The melting points of  $\text{AsCl}_3$ ,  $\text{AsBr}_3$  and  $\text{AsI}_3$  are  $-13^\circ\text{C}$ ,  $31^\circ\text{C}$ . and  $146^\circ\text{C}$ . respectively and the heats of vaporisation of  $\text{AsCl}_3$  and  $\text{AsI}_3$  are 6.7 and 19.2 kcal respectively. From this it can be seen that the latent heat of sublimation of  $\text{AsBr}_3$  will be approximately 10 kcal and  $\frac{R}{3}$  will, therefore, be approximately equal to 47 kcal.

Similarly, the value of  $\frac{R}{3}$  in the case of  $\text{PI}_3$  is approximately equal to 34 kcal. The values of the energy of optical dissociation of these salts are equal to 87.6 and 87.4 kcal respectively. The difference between the optical and thermochemical values is very large. In the case of  $\text{AsBr}_3$  the optical value roughly equals  $\frac{2}{3}R$ . If this equalisation be considered and Saha and Datta's hypothesis<sup>2</sup> be taken to be true, we should consider it to be a case in which two bromine atoms have been knocked off simultaneously by the incoming radiation. This explanation appears to be improbable because the dislodging of one bromine atom from  $\text{AsBr}_3$ , whose probability is greater and which should manifest itself by another absorption did not take place. In the case of  $\text{PI}_3$  things were just the same. Here the value of the energy of optical dissociation was a good deal more than  $\frac{2}{3}R$  and as in the previous case no other absorption could be traced. This anomaly cannot, therefore, be attributed to the knocking off simultaneously of two halogen atoms from the molecule by the incident radiation.

It was found, in addition, that there are some compounds for which the long wavelength limit of absorption varies as we increase the temperature of the substance by fairly wide degrees. There are others for which the variation is very little. In the case of  $\text{SbBr}_3$ , for instance, after the substance had been heated to  $250^\circ\text{C}$ . the long wavelength limit of absorption does not further shift towards the red with greater increase in its temperature. In the case of others the shift towards the red takes place regularly as the temperature goes on increasing. The shifts are not alike in all the cases. In the case of  $\text{SbCl}_3$  and  $\text{AsI}_3$ , for example, the shifts are large at lower temperatures, but go on reducing as the temperature increases. In the cases of  $\text{AsBr}_3$  and  $\text{PI}_3$  they are equally large all over.

It is to be noted that in the case of  $\text{SbBr}_3$ , where there is no shift above about  $250^\circ\text{C}$ ., the values of the energy of optical dissociation and of  $\frac{R}{n}$  are nearly equal. In the case of  $\text{SbCl}_5$  and  $\text{AsI}_3$ , where the shifts go on reducing with the increase in temperature, the difference between the energy of optical dissociation and  $\frac{R}{n}$  is small, as compared to  $\frac{R}{n}$ . In the case of  $\text{AsBr}_3$  and  $\text{PI}_3$ , where the shifts remain the same all over the range of temperature, the difference between the two values is large as compared to the values of  $\frac{R}{3}$ .

These facts are very significant. Unfortunately, in these compounds heating brings about both a change in the temperature of the gas and a change in the pressure of the absorbing column. Experiments with hydrogen halides are being conducted where this ambiguity between the temperature and pressure effect could be eliminated at will. They can still be explained as follows. The transition from the stable electronic level to a higher unstable one takes place in the three cases as represented by Franck Condon diagrams<sup>3</sup> in the figures (a), (b) and (c).

In case (a) the upper level is disposed as shown in the figure. When the transition takes place from the lowest vibrational level of the stable electronic state it goes up to the higher state at a point where it is not horizontal. With the increase in the vibrational level in the lower state, brought about by an increase in temperature, there is both a horizontal and a vertical shift in the position of the point of maximum transition probability on the lower curve. The vibrational energy

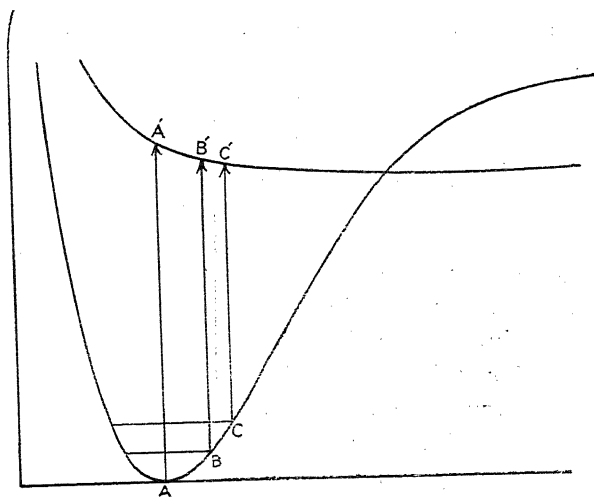


Fig. (a)

being small and the curve itself being not very much steep there, the vertical shift is very small as compared to the horizontal one. But with a horizontal shift in the position of maximum transition probability in the lower curve, the vertical distance as represented by the arrow get considerably reduced because of the slope of the curve. This vertical distance represents the energy of the beginning of absorption. This slope of the upper curve goes on reducing as we

go further to the right. Therefore on a further horizontal shift in the position of maximum transition probability brought about by an increase in the temperature of the substance we go to points having smaller slope on the upper curve and the vertical distance represented by arrows does not change so much. On the upper curve such points with a small slope are only very slightly above the horizontal line asymptotic to it. Since the height of this

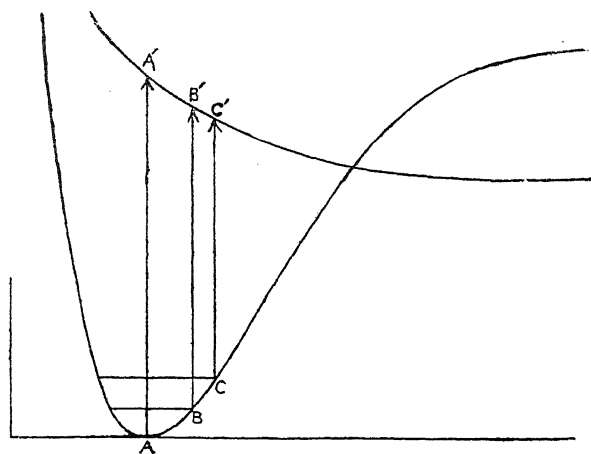


Fig. (b)

asymptote above the lowest vibration level of the lower state gives the heat of dissociation of the salt the value obtained optically is only very slightly greater than the heat of dissociation or it may be equal to or even a little less than the thermochemical value.

In case (b) the upper curve has got a greater slope at all the points to which the transition takes place at various temperatures used in the experiment. The horizontal asymptote is in this case below the most extreme right of these points. The heat of dissociation obtained optically is, therefore, greater than  $\frac{R}{3}$ .

The case (c) is much more extreme. Here at the points to which the transition takes place the upper curve has a far greater slope and the asymptote will be very much below these points. The value of energy of dissociation obtained optically is, there-

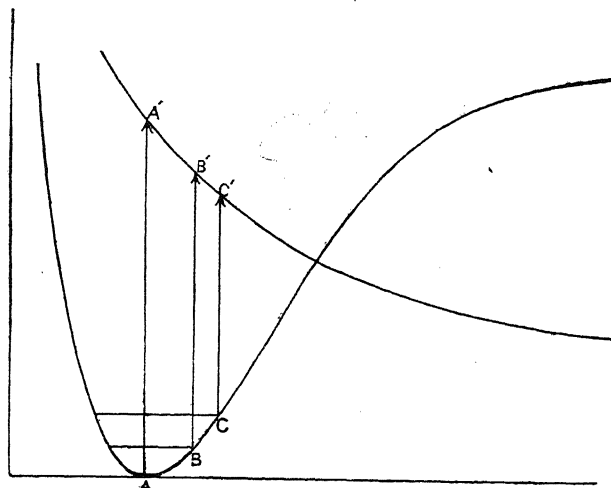


Fig. (c)

fore, much greater than  $\frac{R}{3}$ .

These observations enable us to review the validity of Saha and Datta's hypothesis.<sup>2</sup> We see that in the case of compound  $\text{SbBr}_3$  where the upper Franck Condon curve is practically horizontal at the point of transition and

therefore the long wavelength limit of absorption gives the true value of the energy of optical dissociation, Saha and Datta's hypothesis<sup>2</sup> is found to be valid. In the case of other compounds the limit of absorption does not give the true value of the energy of optical dissociation, for the upper curve has a much larger slope at the point of transition. The greater the deviation of the limit of absorption from the energy of optical dissociation, the more is Saha and Datta's hypothesis found to be invalid. It might be, therefore, very much possible that Saha and Datta's hypothesis is true and, in the very many reports which we have been having regarding its alleged invalidity, the error might have been made in computing the energy of optical dissociation from the long wavelength limit of absorption by disregarding the effect of the slope of the upper curve at the point of transition.

Coming to the third feature of these results it is found that although the salts examined are bromides and iodides, retransmission is to be found only in the case of  $\text{AsI}_3$  and  $\text{SbBr}_3$ . These appear only at rather high temperatures as found in the case of tinhalides.<sup>4</sup> The difference between the energies of an iodine atom in  $^2\text{P}_{3/2}$  and  $^2\text{P}_{1/2}$  states is  $7600 \text{ cms.}^{-1}$ , whereas the difference between these limits in the case of  $\text{AsI}_3$  is only  $2402 \text{ cms.}^{-1}$ . It is obvious, therefore, that this difference is not due to the breaking off of the iodine atom in its two metastable states. As we have seen in the case of tin dichloride<sup>5</sup> the retransmissions can also be due to the breaking off of the molecule into residual molecules in different metastable states. The two absorptions are, therefore, probably, due to  $\text{AsI}_3$  breaking up into iodine and  $\text{AsI}_2$  (normal and excited). In the case of  $\text{SbBr}_3$ , the corresponding difference is  $3390 \text{ cms.}^{-1}$ . The difference between the energies of the normal and excited bromine atoms is  $3700 \text{ cms.}^{-1}$ . The two retransmissions may represent the breaking off from the molecule of a normal and an excited bromine atom in the two cases. But the discrepancy between the two differences warns us not to place much reliance on this explanation. The possible explanation should again be sought in the dissociation of the molecule into Br and two dibromides of arsenic (normal and excited). It is curious that in all such polyatomic molecules retransmissions corresponding to the two metastable levels of bromine and iodine are conspicuous by their absence. An explanation of this is, however, still wanting.

#### ACKNOWLEDGMENTS

It is my pleasant duty to acknowledge my indebtedness to Prof. M. N. Saha, for his extreme kindness on the author and for his immense interest in this work. My heartiest thanks are due to Dr. Asundi of Muslim University, Aligarh, for his having taken the microphotograms for me and to Mr Prabhat Kumar Sen-Gupta for his having carried my plates to Aligarh.



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ON NEW TREMATODES OF FROGS AND FISHES OF THE  
UNITED PROVINCES, INDIA.

**Part III.**—On a new Genus *Mehraorchis* and two new Species of *Pleurogenes* (*Pleurogenetinae*) with a systematic Discussion and Revision of the Family *Lecithodendriidae*.

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Communicated by Dr. H. R. Mehra

Received August 2, 1933

***Mehraorchis ranarum*. Nov. gen., nov. spec.**

Host—*Rana cyanophlyctis*

Habitat—The body cavity, usually the pancreatic region.

Locality—Sitapur, Oudh (India)

These interesting distomes are found enclosed in cysts in the body-cavity of—*Rana cyanophlyctis*—a fairly common frog in the ponds of Sitapur district. The cysts, each containing 2–5 parasites, are usually found in the pancreatic region of the host. The maximum frequency of infection is about 40 per cent and the number of parasites varies from 2–14 in a single host. The non-transparent and sluggish-looking worms are of a dirty brown colour with little power of contraction and expansion. The thick ovoid and slightly convex body is beset with straight bluntly-pointed short spines, 0.013 mm. long and 0.005 mm. thick at the base. The spines at the anterior end are thickly crowded but in the post-acetabular region they are smaller in number and far separated from one another. The spines do not project out of the surface of the body. Sexually mature parasites when alive measure 2.4–4.1 mm. in length and 1.3–2.8 mm. in maximum breadth which occurs in the region a little behind the acetabulum. In fixed specimens the dimensions vary from 2.6–4.1 mm. in length and 1.4–2.9 mm. in breadth. The size of the parasites depends largely upon the number enclosed in a cyst. Usually the cysts contain only two parasites which are bigger in size than those in cysts containing a larger number. The suckers are poorly developed and have a spherical outline. The subterminal and ventrally directed oral sucker is 1.2–1.3 times as large as the acetabulum which lies at the junction of the anterior and the middle thirds of the body. A prepharynx is absent. The

muscular pharynx is conical measuring 0.176 mm. in length with an equally broad base. At times it may protrude slightly into the cavity of the oral sucker. A circular constriction divides the lumen of the pharynx into a shorter anterior and a larger posterior chamber. Posteriorly it leads into a fairly long oesophagus, 0.38–0.56 mm. in length and 0.08–0.12 mm. in breadth, which bifurcates at the end of the anterior one-fourth body length. The two wide intestinal caeca extend up to the broad posterior end of the body. Large amoeboid cells line the lumen of the wide intestinal caeca; their nuclei being situated basely and the distal projecting pseudopodia appear largely vacuolated.

The testes are massive and have a roughly triangular or rhomboidal shape usually with an irregular margin. They are situated right and left in the region bounded on each side by the oesophagus, the intestinal caecum and the body-wall. The right testis, 0.56–0.76 × 0.32–0.54 mm. in dimension, is placed a little in advance of the left which is pushed posteriad by the presence of a well-developed cirrus sac situated in front on the same side. The left testis is usually a little smaller than the right and measures from 0.42–0.8 mm. in length and 0.27–0.43 mm. in maximum breadth. The vas efferens of the right testis crosses in front of the ventral sucker to the left side and runs forwards till it unites with the small vas efferens of the left testis to form a rather inconspicuous vas deferens which enters the large and coiled vesicula seminalis. The latter is an inverted U-shaped structure, with a constriction in the middle, occupying the posterior two-fifths of the cirrus sac. The cirrus sac, measuring 0.48–0.6 × 0.12–0.24 mm., is a well-developed, broad, bluntly-pointed, spindle-shaped structure with its extremities slightly curved in opposite directions. It lies obliquely anterior to the left testis extending behind and lying ventrally up to the commencement of the left intestinal caecum. The vesicula seminalis passes anteriorly through a small narrow duct into an elongated conical pars prostatica measuring 0.2–0.3 × 0.05–0.01 mm. Throughout its length the pars prostatica is surrounded by numerous prostate gland cells with prominent rounded nuclei. The anterior curved and narrow part of the cirrus sac encloses a fairly long ductus ejaculatorius which opens into the genital atrium ventrally to the vaginal pore. The small knob-like cirrus is not covered with spines.

A massive ovary of variable shape usually with a markedly crenated outline lies partly overlapping the ventral sucker in the space between the latter and the right intestinal caecum. It measures from 0.26–0.5 mm. in length and 0.26 to 0.45 mm in breadth. A small elongated bulb-shaped receptaculum seminis is situated to the left side of the ventral sucker. The Laurer's canal arises from the neck of the receptaculum seminis just before it opens into the oviduct. The oviduct dilates to form the ootype where it is surrounded by a diffuse mass of shell gland cells. The common vitelline

duct forms a small triangular yolk reservoir which opens into the oviduct immediately before it passes into the ootype. The uterus first runs parallel to the receptaculum seminis and then continues downwards in a more or less straight course in the median line almost parallel to the length of the body. This descending portion of the uterus is filled with sperms and is to be regarded as receptaculum seminis uterinum. The ascending part of the uterus lies in convoluted transverse windings which posteriorly do not extend behind the bifurcation of the excretory bladder; and anteriorly beyond the ovary and the vitellaria. Laterally, however, they extend outside the intestinal cæca reaching almost the body margins. In one specimen in my collection the uterine coils are seen extending as far as the extreme hinder end but this condition is probably due to too much pressure to which the specimen was subjected during fixation. The uterine coils are mostly confined to the ventral half of the body. In its terminal portion the uterus runs straight in an oblique direction from the ventral sucker to join a feebly muscular metraterm which opens into the shallow genital atrium. The vaginal pore is seen lying dorsal to the male genital opening on the left body margin in a slight depression—"the genital atrium." The whole uterus is packed with innumerable eggs of a deep brown colour. The eggs are operculate and measure  $0.03-0.33 \times 0.013-0.015$  mm. The easily eversible shallow genital atrium lies in level with the pharynx.

The vitellaria of the two sides lie laterally confined to the ventral surface of the body underneath the testes and the intestinal cæca, extending slightly inward towards the median line but not meeting each other. The right vitelline gland lies a little anterior to that of the left side, extending from the middle of the oesophagus to a little distance behind the posterior margin of the acetabulum. Inwardly also it spreads further towards the median line partly covering the ovary. The left vitelline gland occupies the region from behind the obliquely placed cirrus sac to a short distance behind the ventral sucker. The vitellaria never extend backwards to overlap the uterine coils. Each gland consists of a number of closely scattered bunches of grapelike follicles. Small vitelline ducts arise from these groups and fuse to form one large duct on each side. The vitelline duct of each side runs towards the median line and unites with its fellow of the other side near the ventral sucker to form a yolk reservoir which, as mentioned before, opens into the oviduct through a small common vitelline duct.

The excretory bladder is somewhat Y-shaped with the stem of a smaller length than the cornua; its shape approaches somewhat midway between that of latters Y and V. The main stem bifurcates anteriorly at  $0.32-0.48$  mm. from the posterior end. It measures  $0.27-0.43$  mm. in length and has a funnel-shaped outline with the greatest breadth of  $0.16$  mm. at its anterior margin. At its anterior corners arise the cornua, one on each side, extending

as far forward as the ventral sucker. The terminal part of the main stem is surrounded by deeply staining parenchymatous cells which form a sphincter around the opening. The excretory opening lies subterminally at the posterior end on the ventral side of the body.

This interesting parasite is assigned to the subfamily *Pleurogenetina* Looss as defined by Mehra and Negi 1928, with which it presents unmistakable affinities on account of the topography of the genital organs and the position of the genital pore on the left body margin. The excretory bladder, though somewhat different, can, nevertheless, be derived from the typical V-shaped condition characteristic of the subfamily, by the union of the arms for some length forwards to form a moderately long main stem. Amongst the known genera of the *Pleurogenetinae* it resembles *Pleurogenes* in the form of the body, the relatively great length of the intestinal cæca and its occurrence in cysts (*P. arcanus* Klein 1905 occurs encysted in the liver, pyloric region and neck of urinary bladder of frogs). It resembles somewhat the genus *Prosotocus* in the position of its testes which lie one on each side in the anterior part of the body, but the left testis, however, lies behind the cirrus sac and not in front of it as in *Prosotocus*. The vitellaria are confined, as in the genus *Ganeo*, to the ventral half of the body. In all other important features this species does not fit in with any of the known genera, therefore, a new genus is created for its reception. The genus is named after Dr. H. R. Mehra to whom my respectful gratitude is due, for his invaluable help and advice.

*Generic Diagnosis*—*Pleurogenetinae*, small, fleshy, ovoid with flattened elliptical cross section; integument spiny, spines sparsely distributed in post-equatorial region. Suckers small, slightly muscular, acetabulum smaller than the oral sucker lying at the junction of anterior and middle thirds of body-length. Prepharynx absent; pharynx well-developed and muscular; oesophagus moderately long; intestinal cæca extending nearly up to the posterior end. Testes massive; asymmetrically situated in the anterior body to the right and left in the space between oesophagus, intestinal cæca and body-wall. Ovary to the right side, partly overlapping the ventral sucker. Vitellaria, confined to the ventral half in the anteriolateral region extending almost to the median line, composed of a large number of grape-like bunches. Laurer's canal and receptaculum seminis present; shell gland diffuse, situated close to the acetabulum. Genital atrium shallow, easily eversible; situated on a level with the pharynx on the left body margin; male and female openings separate. Excretory bladder with a somewhat prominent stem and longer cornua; excretory pore subterminal and ventral. Muscular cirrus sac situated obliquely, anterior to left testis; containing well-developed coiled vesicula seminalis, pars prostatica and ductus ejaculatorius. Uterus post-ovarian, not extending behind blind ends of intestinal cæca; metraterm present. Ova

numerous small, operculate, measuring  $0.03-0.033 \times 0.013-0.015$  mm. in size.

Encysted in the body cavity, especially in the pancreatic region of Amphibia.

### Genus *Pleurogenes*, Looss, 1896

This is the oldest genus belonging to the subfamily *Pleurogenetinae* Looss 1899, the type species of which was described by Rudolphi in 1819 as *Distoma clavigerum*, parasitic in the intestine of Anurans. V. Linstow in 1888 described the same parasite under the name *Dist. neglectum*. Pagenstecher in 1857 and Pachinger in 1888 described with figures a distome similar to that described by Rudolphi and gave it the same name. Olsson described the specimens of *Dist. medians*, which he found in the intestine of Anurans in 1876. Sonsino also described the same form in 1894 as *Dist. tacapense*, which Looss in 1898 renamed as *Dist. tacapensis*. Looss in his well-known paper published in 1899 pointed out the identity of *Dist. clavigerum* Rud. with *Dist. neglectum*, V. Linstow and of *Dist. medians* Ols. with *Dist. tacapense* Sons and *Dist. tacapensis* Lss. The only representative of the genus *Pleurogenes*, parasitic in the Reptilia was described in 1896 independently by Looss and Sonsino, as *Dist. tacapense* from the intestine of *Chamaeleo basiliscus*. Two years later Looss gave this species its present name of *Pleurogenes tener* and in the following year transferred it to the genus *Prosotocus*. Stafford in 1905 following Looss retained it under *Prosotocus*, but Klein, in the same year, however, transferred it back to the genus *Pleurogenes*, adding two more new species to the genus, i.e. *P. sphericus* from the intestine of an Indian frog *R. hexadactyla* and *P. arcanus* found encysted in the liver, pyloric region and around the neck of the urinary bladder of some members of *Ranidae*. The latter species was first described by Stafford in 1900 as *Dist. medians* Ols. and almost at the same time by Nickerson as *Dist. arcanum*. Four years later Stafford created a new genus *Loxogenes* for *Dist. arcanum*. Mehra and Negi in 1928, following Klein, have referred it to the genus *Pleurogenes* in their key. Tubangui in 1928, Fuhrmann in 1928, Travassos in 1930 and Krull in 1933 have, however, retained the genus *Loxogenes*. As will be seen from the discussion the genus *Loxogenes* cannot be maintained. *P. gastroporus*, parasitic in the intestine of *R. cyanophlyctis* India, was described by Luhe in 1901 and a new variety of it *var. equalis* from the intestine of *R. tigrina* was added by Mehra and Negi in 1928. Johnston in 1912 described *P. freycineti* and *P. solus* from the intestine of Australian tree frogs, *Hyla freycineti* and *H. aurea*. Travassos in 1921, following the suggestion made by Stafford in 1904 split up the genus *Pleurogenes* Lss. into two groups on the basis of the length of the intestinal cæca. Group one, comprising of the species with intestinal cæca extending beyond the ventral

sucker he retained as the genus *Pleurogenes*; whereas for the species in which the cæca are short, never extending beyond the centrum of the acetabulum, he created a new genus *Pleurogenoides*. Mehra and Negi in 1928 divided the genus *Pleurogenes* Lss. into two sub-genera—*Pleurogenes* and *Telogonella*—on the basis of the length of the intestinal cæca and the position of the genital pore. In the sub-genus *Pleurogenes* the intestinal cæca do not extend posteriorly beyond the centrum of the acetabulum and the genital pore is more cephalad, while in *Telogonella* the cæca extend posteriorly beyond the acetabulum and the genital pore is not so anteriorly situated. In his papers published in 1930 and 1931 Travassos reaffirms the validity of his genera *Pleurogenes* and *Pleurogenoides*. The study of intermediate species between the two genera created by Travassos, such as *P. orientalis* n. sp., *P. lobatus* Ozaki 1926 and *P. intermedius* Isaitschikow 1926 (Travassos did not consult this paper) has convinced me of the untenability of the genus *Pleurogenoides* Trav., which I accordingly drop, referring all its species back to *Pleurogenes* Looss. Ozaki in 1926 described *P. lobatus* from the bile ducts of Japanese frogs—*Polypedates buergeri*. Two years later Tubangui found a species *P. taylori* in the intestine of *R. vittigera* in Philippine. In 1930 Travassos described *P. stromi* from the intestine of *R. esculanta* and Africa *P. loossi* from the intestine of the same host. The latest addition to the list of species of this genus is *P. minus* parasitic in the intestine of Pike—*Esox lucius*, described by Pigulewsky in 1931. I add in this paper two new species to this genus from the gut of *R. cyanophlyctis* (Northern India).

***Pleurogenes orientalis*.—n. sp.**

Host—*Rana cyanophlyctis*.

Habitat—Intestine.

Locality—Sitapur, Oudh (India).

In its frequency of occurrence this species is as rare as *Prosotocus infrequentum* Srivastava 1933. In August 1932 only two frogs were found infected with one parasite each, out of over a hundred specimens of *R. cyanophlyctis* examined. The distomes show little power of contraction and expansion and are of a light brown colour. The thin and transparent body is ovoid in form with broadly rounded ends and is studded with minute backwardly directed spines except in the region behind the excretory opening. In balsam mounts the parasites measure 1.4–1.6 mm. in length and 0.96–1.1 mm. in maximum breadth across the acetabular region. The suckers are fairly large and muscular with spherical outline. The oral sucker, 0.27–0.28 mm. in diameter, is larger than the acetabulum and is situated on the ventral surface a little behind the anterior end. The acetabulum lies for the greater part of

its diameter in the posterior half of the body measuring 0.22 mm. in diameter. The size ratio of the oral and the ventral suckers is 4:3.

The genital opening is situated on the left body margin in level with the junction of the anterior and middle thirds of the oral sucker. The excretory pore is subterminal, situated on the ventral surface a little in front of the hinder end. A muscular pharynx,  $0.064 \times 0.012$  mm. in size, lies at the base of the oral sucker. The oesophagus being entirely absent the pharynx is immediately followed by the intestinal bifurcation which lies 0.3 mm. in front of the acetabulum. The intestinal caeca at first run more or less horizontally, one on each side and then continue their downward course, laterally near the body-wall, terminating a little behind the acetabulum, just in front of the posterior third of body length.

The testes lie symmetrically near the lateral body-walls, one on each side, immediately behind the blind extremities of the intestinal caeca about the junction of the middle and posterior thirds of the body length. The right testis is nearly spherical in shape, measuring 0.19 mm. in diameter, while the left testis,  $0.14 \times 0.17$  mm. in size, is more or less ovoid in outline. The vasa efferentia, which arise as delicate tubes from the anterior margin of each testis, run forwards separately for a short distance before they unite in the median line close in front of the acetabulum to form a small vas deferens which soon enters the cirrus sac. The cirrus sac is well developed and situated to the left side, ventrally to the left intestinal caecum, extending from the anterior margin of the acetabulum to the level of about anterior one-third diameter of the oral sucker. It is quite large for the size of the distome, measuring 0.72 mm. in length, and consists of a basal horizontal club-shaped part and a narrow tubular vertical part. The angle of curvature between these two parts is a sharp right angle. The basal club-shaped part encloses a coiled vesicula seminalis of 0.32 mm. length and a fairly long pars prostatica which becomes narrower towards its terminal end, where it passes into the ductus ejaculatorius of 0.3 mm. length. The cirrus sac opens terminally into the inconspicuous genital atrium which lies on the left body margin.

The ovary, 0.13 mm. in diameter, is spherical and is situated to the right side about the end of the first body-half, close inside and in contact with the right caecum, at about the level of the anterior half of the acetabulum. The oviduct arises from the middle of its inner margin and is joined after a short length by a flask-shaped receptaculum seminis of  $0.13 - 0.15 \times 0.08 - 0.1$  mm. size which lies obliquely to the right side of the acetabulum between it and the ovary. As in the other species of *Pleurogenetinae* a small Laurer's canal arises from the receptaculum seminis. The yolk reservoir, the shell gland mass and the receptaculum seminis are all lodged in the space between the ovary and the acetabulum.



The vitellaria are well developed and confined to the anterior half of the body. The follicles are scattered mesially between the oral sucker and the acetabulum, running into one another at a few places, but they are much aggregated laterally extending from the middle of the oral sucker to the level of about the middle of the acetabulum.

The uterus arises from the right side of the acetabulum, passes downwards and forms in the post-acetabular region a compact convoluted mass filling a little more than the posterior one-third of the body. The ascending part of the uterus passes distally to the left side of the acetabulum, running parallel and outer to the cirrus sac, before it terminates into a feebly muscular metraterm. The metraterm opens in the inconspicuous genital atrium to the left side of the male opening. The uterus is packed with numerous small operculate eggs of a light brown colour measuring  $0.023 \times 0.013$  mm. in size.

The excretory bladder is V-shaped with its cornua extending anteriorly up to the posterior margin of the testes. The excretory pore lies on the ventral surface, 0.13 mm. distance in front of the posterior end of the body.

This interesting species differs remarkably in many features from all the other species of the genus *Pleurogenes* Lss. It resembles somewhat *P. gastroporus* and *P. gastroporus* var. *equalis* in the shape of its body, shape of the excretory bladder, position of the excretory pore and the uterine convolutions being confined to the post-acetabular region. But it differs from them in such important features as the relative position and size ratio of the suckers, absence of the œsophagus, relative length of the intestinal cæca, shape and disposition of the vitellaria, topography of the gonads, the shape and position of the cirrus sac and the position of the genital pore.

#### ***Pleurogenes sitapurii*.—n. sp.**

Host—*Rana cyanophlyctis*.

Habitat—Duodenum.

Locality—Sitapur, Oudh (India).

The parasites belonging to this species are the smallest of all the distomes infecting *Rana cyanophlyctis*. They were first met with about the middle of July 1932. During the rainy season (July—September) the frequency of infection is about 40 per cent varying in intensity from 6—25, but with the approach of winter it gradually declines. The parasites are usually found attached to the wall of the descending part of the duodenum. Only once they were found throughout the length of the duodenum. In their natural habitat they appear as minute dust particles, rendered conspicuous by the colour of the contained ova. They are so delicate that they can hardly bear the weight of even a small glass coverslip. They appear to be extremely susceptible to changes in diet and temperature, as they cannot live for more than an hour in any

nutritive solution. In the living condition they are grey in colour and show little power of contraction and expansion, measuring 0.78–0.94 mm in length and 0.46–0.64 mm. in maximum breadth which lies across the acetabulum. The body is comparatively thick and presents an oval outline, narrower anteriorly and broader and somewhat rounded off behind. In entire mounts the size varies from 0.6–0.96 mm. in length and 0.35–0.5 mm. in maximum breadth, according to the state of contraction. The cuticle is covered all over with fairly large and pointed spines of 0.013 mm. length.

The suckers are feebly muscular and have a circular outline. The oral sucker is subterminally situated on the ventral surface, measuring 0.11–0.13 mm. in diameter. The acetabulum, 0.11–0.14 mm. in diameter, *i.e.*, nearly equal to or slightly larger than the oral sucker, is situated in the posterior half of the body, just in front of the hinder third of body length.

The genital atrium, containing the male and the female openings, is situated on the left body margin in level with the pharynx. The excretory opening is situated on the ventral surface a little in front of the posterior extremity.

The muscular globular pharynx of 0.03–0.065 × 0.05–0.07 mm. size is followed by a short oesophagus of 0.06–0.1 mm. length which bifurcates just in front of the ovary, in level with the junction of the pars prostatica and the ductus ejaculatorius. The intestinal cæca, usually of equal length, are short with moderately divergent extremities, ending blindly much in front of the acetabulum. They rarely extend beyond the posterior ends of the ovary and the cirrus sac.

The testes are massive structures, rounded or ovoid in shape, situated somewhat asymmetrically one on each side immediately in front of the centrum of the acetabulum. The right testis, 0.1–0.23 × 0.08–0.18 mm. in size, is usually a little larger than the left testis and is separated from the right body margin by the anteriorly passing coils of the uterus. The left testis, 0.1–0.23 × 0.08–0.1 mm. in size, is pushed somewhat caudad by the well-developed cirrus sac which lies in front and is separated from the latter and the acetabulum by the outgoing coils of the uterus. The vasa efferentia arise as delicate tubes, one from the posterior inner end of each testis, and run more or less transversely in front of the acetabulum to enter the vesicula seminalis through a small and inconspicuous vas deferens. The cirrus sac, 0.3–0.36 × 0.06–0.09 mm. in size, is highly muscular and has a slight S-shaped curvature. The coiled vesicula seminalis is divided by a prominent constriction into two distinct parts, *i.e.*, a basal swollen sac-shaped part of 0.11 × 0.08 mm. size and an anterior tubular part of 0.08 mm. length and 0.02 mm. breadth. The pars prostatica is elongated and somewhat flask-shaped, measuring 0.1–0.16 × 0.03–0.04 mm. in size. It narrows anteriorly to form the ductus ejaculatorius of 0.06–0.1 mm. length which is followed by a knob-like cirrus of 0.013 mm. length.

The ovary,  $0\cdot09-0\cdot18 \times 0\cdot05-0\cdot13$  mm. in size, is situated somewhat in the median line, more to the right side, far in front of the acetabulum and close behind the intestinal fork partly overlapping the right intestinal cæcum. Usually it has a regular outline with entire margin but sometimes it is lobed. The receptaculum seminis,  $0\cdot1 \times 0\cdot04$  mm. in size, is flask-shaped, situated transversely or obliquely, to the right side, close in front of the acetabulum. The Laurer's canal,  $0\cdot02$  mm. in diameter, arises as a curved tube from the neck of the receptaculum seminis and runs posteriorly to open on the mid-dorsal surface in the region of the acetabulum.

The vitellaria are composed of usually an equal number of 4–8 large, pear-shaped follicles, each of  $0\cdot05-0\cdot09 \times 0\cdot02-0\cdot05$  mm. size. The left vitelline gland lies close to the median line, behind the intestinal bifurcation, in the space between the cirrus sac and the ovary. The right vitelline gland lies close to the right body margin and slightly cephalad to the left gland. The two vitelline ducts run posteriorly and unite together to form the common vitelline duct to the right side of the ventral sucker. The common vitelline duct travels forward to form the yolk reservoir situated in front near the junction of the receptaculum seminis with the oviduct, in the space between the ovary and the acetabulum. A diffuse shell gland mass lies median, just in front of the acetabulum.

The descending part of the uterus commences near the left side of the acetabulum and forms several longitudinal and transverse loops, extending from the left testis to the posterior end of the body. Behind the acetabulum it passes into a transverse loop which extends forwards to the right side in the form of a longitudinal loop right up to the pharynx and then turns downwards forming two or three loops near the hinder end on the same side before it crosses over again to the left side to join the metraterm of  $0\cdot15 \times 0\cdot018$  mm. size, which crosses the cirrus sac to lie close to its right side. In fully mature specimens, the coils of the uterus as described above are indistinguishable and the whole uterus appears as a mass of eggs. The eggs are dark brown in colour, oval and operculate, measuring  $0\cdot023-0\cdot025 \times 0\cdot013$  mm. in size.

The V-shaped excretory bladder consists of two broad cornua and a very small median stem. Both the cornua extend up to the level of the acetabulum and are slightly constricted in the middle of their length. The excretory pore lies on the ventral surface a little in front of the posterior end.

Of all the species of the genus *Pleurogenes* Looss *P. sitapurii* n. sp. is closely allied to *P. solus* Johnston 1912 in the shape and size of its body, position of the suckers and the genital pore and in the vitelline glands being composed of a few large follicles. It differs, however, from *P. solus* in the length of the oesophagus and the intestinal cæca, position and size of the gonads, size and number of the vitelline follicles, characteristic arrangement of the uterine coils and the position of the excretory pore.

### Systematic Discussion on the Genus *Pleurogenes*, Looss, 1896.

The genus *Pleurogenes* Lss. is parasitic in amphibia except *P. tener* which is described from a reptile—*Chamaeleo basiliscus* and *P. minus* which is parasitic in the intestine of a fish—*Esox lucius*. While the position of *Pleurogenes* as the typical genus of the sub-family *Pleurogenetinae* needs no discussion a revision of its scope seems to be necessary.

Stafford in 1904 established the genus *Loxogenes* for *Dist. arcanum* Nickerson 1900 "on account of its genital opening being situated on the ventral surface, midway between the left intestinal caecum and the body margin." This is the only feature which separates *Loxogenes* from *Pleurogenes*. But the genital pore does not lie exactly on the body margin in several other species of *Pleurogenes* such as *P. sphaericus* and *P. intermedius*, in which it occupies a position far inwards to the left body margin. It is, therefore, necessary to drop this genus which contains species closely related to all the other species of the genus *Pleurogenes*.

Travassos in 1921, according to the suggestion made by Stafford in 1904, created a new genus *Pleurogenoides* for such species of the genus *Pleurogenes* as have short intestinal caeca (never extending behind the acetabulum) and retained the genus *Pleurogenes* for *P. claviger*. In his papers published in 1930 and 1931 he has maintained *Pleurogenes* and *Pleurogenoides* as distinct genera. Two genera can be accepted only so long as the generic differences between them are of absolute value and the intermediate forms connecting them do not exist. As the genera *Pleurogenes* and *Pleurogenoides* are now connected by such intermediate species as *P. orientalis* n. sp. *P. intermedius* Isaitschikow and *P. lobatus* Ozaki it appears necessary to drop the genus *Pleurogenoides*. I accordingly drop the genus *Pleurogenoides* and assign the species belonging to it back to the genus *Pleurogenes* Looss.

In splitting up the genus *Pleurogenes* into two subgenera Mehra and Negi have recognized the differences between the two groups of species, without losing sight of their close relationships. It is certainly a convenient arrangement which provides for a systematic grouping of a large number of species belonging to one genus. It may be pointed out that *P. orientalis* n. sp. is one of the intermediate species which connects the two subgenera *P. 'Pleurogenes'* and *P. (Telogonella)*.

In the light of our recent knowledge of the genus the diagnosis of *Pleurogenes* as given by Mehra and Negi needs a certain amount of modification.

The amended diagnosis is as follows:

*Diagnosis*.—Body oval, elliptical, oblong or somewhat spherical; size small; acetabulum usually situated about the middle of body rarely in front or behind it. Oesophagus absent, short or long; length of intestinal caeca

extremely variable. Genital pore situated on ventral or dorsal surface, near or on the left (rarely dextral) body margin, in front of or in level with intestinal bifurcation except in *P. lobatus* where it is behind it. Testes two, regular, except in *P. lobatus* (lobed), situated symmetrically, one on each side, or obliquely one behind the other, in level with, in front of or behind the acetabulum. Ovary regular or lobed, usually pretesticular rarely in level with the testes, dextral or median, near or in front of the acetabulum. Cirrus sac enclosing coiled vesicula seminalis and well-developed pars prostatica present. Vitelline follicles confined to the anterior body half. Uterine convolutions usually confined to the posterior half, rarely extending up to the level of the pharynx. Excretory bladder V-shaped with or without a short median stem or Y-shaped with the main stem much longer than the cornua as in *P. bicolor* Krull 1933; excretory opening terminal or subterminal. Eggs small, and numerous, measuring,  $0.02-0.037 \times 0.01-0.016$  mm. in size.

Host—usually amphibia only exceptionally fishes and reptile

Habitat—the gut of the host except *P. arcannum* which lives in cysts around the pylorus, surface of liver and neck of the urinary bladder of frogs.

### Key to the Sub-genera of *Pleurogenes*, Looss 1896.

- Intestinal cæca confined to the first half of body  
and not extending behind the acetabulum ... Sub-genus *Pleurogenes*.
- Intestinal cæca extending behind the acetabulum  
into the posterior half of body ... Sub-genus *Telogonella*.

### Key to the Species of the Sub-genus *Telogonella*

1. The main stem of the excretory bladder much longer than the cornua ... *P. bicolor*.  
The main stem of the excretory bladder much smaller than the cornua ... 2
2. Gonads lobed ... *P. lobatus*.  
Gonads with entire margins ... 3
3. Testes obliquely situated, one behind the other ... *P. loossi*.  
Testes symmetrically situated, one on each side ... 4
4. Testes overlapping the cæca and situated a little in front of their blind ends ... *P. intermedius*.

- Testes situated behind the termination of the intestinal cæca ... .. 5
5. Intestinal cæca end a little behind the acetabulum, in front of the posterior third body-length ... .. *P. orientalis*.
- Intestinal cæca extend far behind the acetabulum, never ending in front of the  $\frac{2}{3}$ th bodylength ... .. *P. claviger*.

### Key to the Species of the Sub-genus *Pleurogenes*

- Acetabulum distinctly post-equatorial ... A
- Acetabulum equatorial or pre-equatorial .. B
- A. Oesophagus absent ... .. *P. sphericus*.
- Oesophagus present ... .. 1
1. Intestinal cæca extend up to the acetabulum *P. solus*.
- Intestinal cæca do not extend up to the acetabulum ... .. 2
2. Excretory pore terminal ... .. *P. tener*.
- Excretory pore subterminal ... .. *P. sitapurii*.
- B. Ovary extra-cæcal ... .. 1
- Ovary not extra-cæcal ... .. 2
1. Intestinal cæca extend up to the acetabulum *P. minus*.
- Intestinal cæca stop in front of the acetabulum *P. medians*.
2. Oesophagus present ... .. 3
- Oesophagus absent ... .. 4
3. Genital atrium opens on the left body margin *P. freycineti*.
- Genital atrium opens subterminally half way between the left intestinal cæcum and body margin ... .. *P. arcanum*.
4. Testes situated anterior to the ends of the intestinal cæca ... .. *P. taylori*.
- Testes lie behind the ends of the intestinal cæca 5
5. Vitellaria consist of a few large follicles; pre-cæcal ... .. *P. stromi*.
- Vitellaria consisting of a large number of small follicles scattered all over the cæca and meeting in the median line ... 6
6. Acetabulum larger than the oral sucker ... *P. gastroporus*.
- Acetabulum of the same size as the oral sucker ... .. *P. gastroporus* var. *equalis*.

### Systematic Discussion and Revision of the Family Lecithodendriidae. Odhner 1911.

Looss in 1896 created the subfamily *Lecithodendriinae* to include the genera *Lecithodendrium*, *Phanaropsolus* and *Pycnoporos*. Later in 1899 he considered the resemblance between *Lecithodendrium* and *Brachycaelium* to be so close that he dropped the subfamily *Lecithodendriinae* and assigned all the genera to the subfamily *Brachycaeliinae* Looss. Luhe in 1909 adopts the latter course in the "Susswasserfauna Deutschlands". Two years later Odhner reshuffled the whole arrangement and removed all the genera except *Brachycaelium* from the *Brachycaeliinae* to the *Lecithodendriinae* which then is the *Brachycaeliinae* minus *Brachycaelium*. The *Brachycaeliinae*, containing *Brachycaelium*, was assigned by him to the family *Dicrocoelidae* Cort 1919, Mehra and Negi 1926 and Mehra 1931 found this position untenable and assigned this subfamily to the family *Lepodermatidae*.

The subfamily *Pleurogenetinae* was established by Looss in 1899 to include the genera *Pleurogenes* and *Prosotocus*. The *Pleurogenetinae* shows unmistakable affinities with the *Lecithodendriinae* in the presence of a V-shaped excretory bladder, position of the acetabulum about the middle of the body, situation of the ovary near it to the right side or about the median plane and the disposition of the uterine convolutions. Further, the absence of a cirrus sac in *Ganeo* brings the *Pleurogenetinae* closer to such genera of the *Lecithodendriinae* as *Lecithodendrium*, *Prosthodendrium*, *Acanthatrium* and *Pycnoporos*, etc. The important differences between the two subfamilies lie in the position of the genital pore and the host. Odhner in 1911 recognised the close affinities between the two subfamilies and brought them together under one and the same family *Lecithodendriidae*. Though this view of the relationship has never been questioned, the recent work of Fuhrmann in 1928 and of Travassos in 1922, 1928, 1930 and 1931 has caused some confusion in the limits of the two subfamilies owing to the unnatural grouping of the genera under them. Unfortunately both these authors have not given any indication of the reasons for assigning the various genera to the two subfamilies.

Fuhrmann 1928 includes under *Lecithodendriinae* the genera *Lecithodendrium*, *Pycnoporos*, *Phanaropsolus*, *Parabascus*, *Mesodendrium*, *Acanthatrium*, *Limatulum* and *Castroia*, and under the *Pleurogenetinae* the genera *Pleurogenes*, *Loxogenes*, *Prosotocus*, *Mosesia*, *Postorchigenes*, *Brandesia*, *Pleurogenoides*, *Ganeo*, *Eumogacetes* and *Anchitrema*. Travassos in 1922 while describing a new species of *Eumagacetes*—*E. perodiosus* from the cloaca of *Pioya cajanula*, Brazil—raised the genus to the rank of a family—*Eumagacetidae*. In 1928 he included the genera *Lecithodendrium*, *Paralecithodendrium*, *Acanthatrium* and *Castroia* under *Lecithodendriinae*. In 1930 under the *Pleurogenetinae* he included the genera *Pleurogenes*, *Pleurogenoides*, *Cryptotrema*, *Limatulum*, *Loxogenes*, *Prosotocus*

*Brandesia*, *Phanaropsolus*, *Mosesia* and *Parabascus*. In the last paper he also referred the genus *Ganeo* to the subfamily *Lecithodendriinae*.

From the above lists it appears that the classification of the family *Lecithodendriidae* is entirely arbitrary and that a revision of the family is needed.

The genera *Mesodendrium* Faust 1919 and *Lecithodendrium* Looss 1896 have already been referred as synonymous by Dollfus in 1931. The untenability of the genera *Loxogenes* Stafford and *Pleurogenoides* Travassos has been made clear in the systematic discussion before and the genus *Ganeo* has been already assigned by me 1933 to the *Pleurogenetinae*. The family *Eumagacetidae* Travassos 1922 based on the length of the intestinal cæca and the extent of the vitellaria—characters which vary within wide limits in the family *Lecithodendriidae*—cannot be maintained. I, therefore, refer the genus *Eumagacetes* to the *Lecithodendriinae* in which it was first included.

The classification of the genera of the *Lecithodendriidae* into subfamilies should not be based on (a) the length of the intestinal cæca, (b) the extent of the vitellaria, and (c) the position of the testes because they are very variable in this family. The only features which show constancy and have little variability are the position of the genital pore and the host. The length of the intestinal cæca varies widely in such closely related genera as *Eumagacetes*, *Lecithodendrium*, *Prosotocus*, *Mehraorchis* and *Pleurogenes* and even in the different species of the last genus; so does also the extent of vitellaria in *Lecithodendrium*, *Prosotocus*, *Prosthodendrium*, *Eumagacetes*, *Pleurogenes*, *Mehraorchis* and *Cryptotropa*. The position of the testes also presents the same condition in different genera. But the position of the genital pore, sinistral, dextral or median and the host are the features which should be recognised as the basis of the division into the two subfamilies. Those genera which have the genital pore situated to the left (exceptionally to the right) of the median line either in front or in the neighbourhood of the acetabulum and are usually parasitic in fishes, amphibia and reptiles are separated under the subfamily *Pleurogenetinae* and the others which have the genital pore situated in or about the median line and are parasitic in reptiles, birds and mammals are included under the *Lecithodendriinae*. The genus *Parabascus* which has the genital pore to the left side and is parasitic in bats combines the characters of both the subfamilies. In this case I should point out that the nature of the host should not be considered of very great importance, as it is probable that the insect larvæ through which the larval stages of the members of the *Lecithodendriidae* are passed, form the food common to hosts of different groups such as amphibia and bats. For similar reasons Braun also cautioned against attaching undue importance to widely different hosts of closely related species, i.e., *Crepidostomum laureatum* Zedar, parasitic in fish and *C. mitocetus*, parasitic in bats.



In view of the several new forms which have been described in recent years the diagnosis of the family *Lecithodendriidae*, as given by Odhner in 1911 needs to be modified and the scope of its subfamilies redetermined.

**Emended Diagnosis of Lecithodendriidae, Odhner, 1911**

Body small rounded or fairly long and elongated; ventral sucker in the middle of the body or not far from it; spines present or absent. Prepharynx present or absent; pharynx present; oesophagus present or absent; intestinal caeca of varying length. Testes situated usually symmetrically, one on each side, rarely obliquely one behind the other in different regions of the body. Cirrus sac present or absent or represented by a pseudocirrus sac. Ovary usually situated to the right side, sometimes in the median line, not far from the ventral sucker; a small receptaculum seminis and Laurer's canal present. Vitellaria of variable extent, consisting of only a few or numerous follicles, confined either to definite regions or scattered all over. Uterus strongly convoluted. convolutions usually confined to the postacetabular region, rarely extending as far forwards as the pharynx; eggs numerous, measuring 0.015–0.06 mm in size. Excretory bladder V-shaped with or without a short median stem, rarely Y-shaped with the main stem larger than the cornua.

Parasitic in insect eating vertebrates, from fishes to mammals.

**Subfamily—Lecithodendriinae, Looss char. emend.**

Body elongated or rounded, moderately large or small, suckers well developed; spines present or absent. Intestinal caeca only exceptionally extend beyond the acetabulum as in *Eumagacetes* and *Anchitrema*. Testes more or less symmetrically situated; genital pore median, situated in front or in the neighbourhood of the acetabulum. Eggs small and numerous, 0.017–0.03 mm. in size. Excretory bladder V-shaped.

Parasitic in reptiles, birds and mammals.

**Subfamily—Pleurogenetinae, Looss, char. emend.**

Body elliptical, elongated, oval or rounded, size small; suckers not particularly muscular; body partly or wholly beset with spines. Intestinal caeca of variable length. Testes variable in position, usually symmetrically situated, one on each side; rarely obliquely placed one behind the other; cirrus sac or pseudocirrus sac present or absent. Genital pore sinistral, rarely dextral, marginal or submarginal; in front or in the neighbourhood of the acetabulum. Vitellaria of varying extent. Uterus much convoluted, generally confined to the postacetabular region, exceptionally extending as far forwards as the pharynx; metraterm present or absent. Excretory bladder V-shaped with or without a short median stem, exceptionally Y-shaped. Eggs usually of deep brown colour, 0.02–0.06 mm. in size.

Parasitic in fishes, amphibia and reptilia except *Parabaszus* which is found in bats.

Family—*Lecithodendriidae* Odhner 1911.

Subfamily—*Lecithodendriinae* Looss 1896.

Genera—1. *Lecithodendrium* Looss 1896.

2. *Pycnoporus* Looss 1899.

3. *Phanaropsolus* Looss 1899.

4. *Anchitrema* Looss 1899.

5. *Eumagacetes* Looss 1899.

6. *Acanthatrium* Faust 1918.

7. *Limatulum* Travassos 1926.

8. *Castroia* Travassos 1928.

9. *Mosesia* Travassos 1928.

10. *Prosthodendrium* Dollfus 1931.

Subfamily—*Pleurogenetinae* Looss 1899.

Genera—1. *Pleurogenes* Looss 1896.

2. *Prosotocus* Looss 1899.

3. *Brandesia* Stossich 1899.

4. *Ganeo* Klein 1905.

5. *Parabascus* Looss 1907.

6. *Cryptotropa* Strand 1931.

7. *Postorchigenes* Tubangui 1928.

8. *Mehraorchis* Srivastava 1933.

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#### EXPLANATION OF PLATES

Fig. 1—Ventral view of *Mehraorchis ranarum*.

Fig. 2—Do. Do. *Pleurogenes orientalis*.

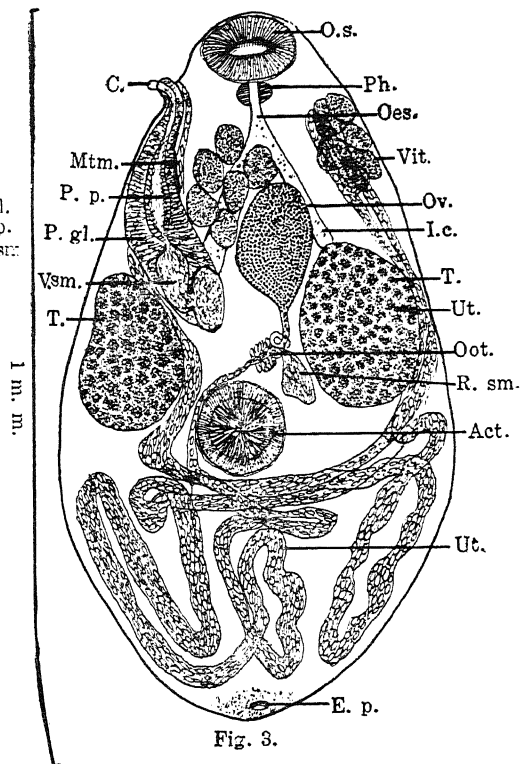
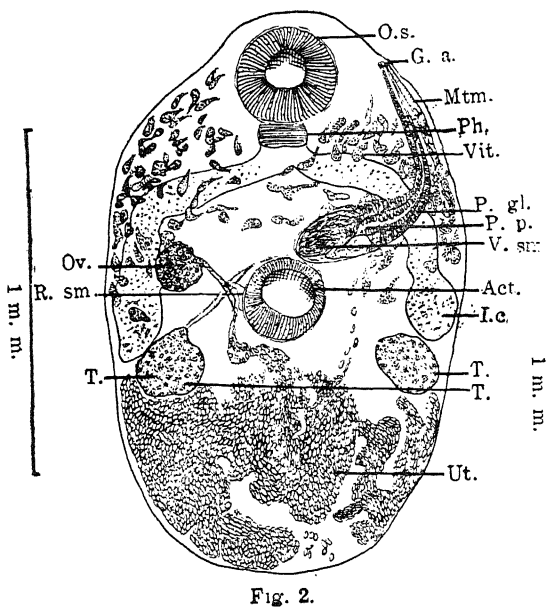
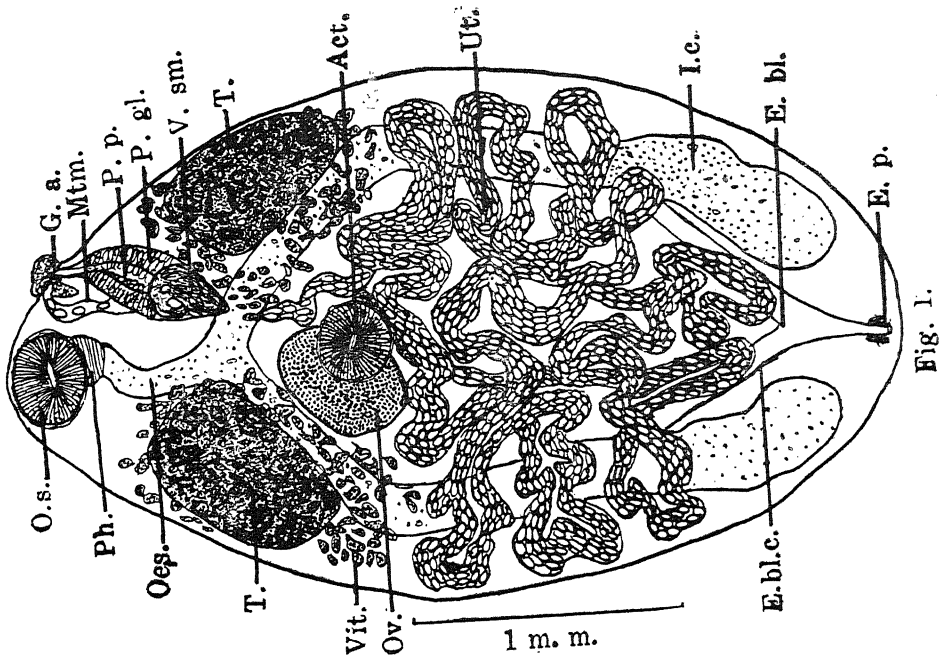
Fig. 3—Dorsal view of *Pleurogenes sitapuri*.

#### LETTERING

Act	...	Acetabulum.	Oes.	...	Oesophagus.
C	...	Cirrus.	Oot.	...	Ootype.
E. bl.	...	Excretory bladder.	O. s.	...	Oral sucker.
E. bl. c.	...	Cornua of Excretory bladder.	Ph.	...	Pharynx.
E. p.	...	Excretory pore.	P. gl.	...	Prostate glands.
G. a.	...	Genital atrium.	R. sm.	...	Receptaculum seminis.
I. c.	...	Intestinal cæcum	T.	...	Testis.
Mtm.	...	Metraterm.	Ut.	...	Uterus.
Ov.	...	Ovary.	V. sm.	...	Vesicula seminalis.
P. p.	...	Pars prostatica.	Vit. d.	...	Vitelline duct.

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# ON THE $\beta$ -RAY ACTIVITY OF RADIOACTIVE BODIES

(Preliminary Communication)

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## Introduction

The  $\beta$ -ray activity of radioactive bodies has up till now proved to be a baffling problem. The points at issue are summarised in Gamow's *Constitution of Atomic Nuclei*, pp. 52—54, and in *Radiations from Radioactive Bodies* by Rutherford, Chadwick and Ellis. They are also discussed at some length by Bohr in his *Faraday Lecture* (Feb. 1932). We shall later quote freely from Bohr, but some fundamental difficulties may be pointed out at the outset.

The older view of the constitution of the nucleus was that it should be regarded as composed of  $A$  protons ( $A$ =mass number) and  $A-Z$  electrons ( $Z$ =nuclear charge). A large number of these protons and electrons may exist in the compound form of  $\alpha$  particles ( $4p+2e$ ) or some other composite structures. But even allowing for these, the existence of a number of free electrons had to be postulated inside the nucleus. On the other hand, the evidence of hyperfine structure, as was first pointed out by de Kronig, definitely proves that the electron cannot exist in the free state in the nucleus, for then the magnetic moment of the nucleus should have the magnitude of the Bohr magneton, while the hyperfine structure of spectral lines definitely shows that the moment has the magnitude of the protonmagnet ( $\frac{1}{1836}$  times the Bohr-magneton). At the present time, it is almost universally held that the nucleus consists of  $Z$  protons, and  $A-Z$  neutrons, but it is quite possible that a number of these are combined in the form of  $\alpha$ -particles, deutons, etc. *The nucleus contains no electrons free or bound.*<sup>1,2</sup>

But this conclusion is seemingly at variance with the observed fact that in  $\beta$ -ray disintegrations the nuclei are observed to eject high speed electrons spontaneously. The situation is therefore paradoxical. Bohr puts it as follows:—

“Strictly speaking, we are not even justified in saying that a nucleus contains a definite number of electrons, but only that the negative

electrification is equal to a whole number of units and in this sense, the expulsion of a  $\beta$ -ray from a nucleus may be regarded as the creation of an electron as a mechanical entity."

In a later passage, Bohr describes the other difficulties as follows :—

"As regards this last question, much theoretical interest has recently been aroused by the peculiar features exhibited by the  $\beta$ -ray expulsions. On the one hand, the parent elements have a definite rate of decay, expressed by a simple probability law, just as in the case of the  $\alpha$ -ray disintegrations. On the other hand, the energy liberated in a single  $\beta$ -ray disintegration is found to vary within a wide continuous range, whereas the energy emitted in an  $\alpha$ -ray disintegration, when due account is taken of the accompanying electromagnetic radiation and the mechanical energy conversion, appears to be the same for all atoms of the same element."

To the above remarks of Bohr, the following may be added :—

(a) The  $\beta$ -ray disintegration has been observed not only in the case of heavy elements, but also in the light elements potassium and rubidium (or rather the isotopes  $K^{41}$  and  $Rb^{87}$ ). In the case of  $\beta$ -ray bodies associated with the main groups (U, Th, Ac), the life of  $\beta$ -ray bodies is found to vary from 16 years (RaD) to a few minutes, but the light elements  $K^{41}$  and  $Rb^{87}$  possess lives comparable with those of some long-lived heavy radioactive bodies. The life of  $K^{41}$  has been estimated to be  $7.5 \times 10^{10}$  years that of  $Rb^{87}$  to be  $10^{11}$  years.<sup>3</sup> It is quite possible that there may be a number of  $\beta$ -ray elements possessing longer lives which are still undiscovered, as the activity of such bodies is likely to be extremely feeble, and difficult of detection. In support of our view, we may cite the case of Ac, RaD . . . which were long regarded as undergoing *rayless* changes. They are not actually rayless, but the  $\beta$ -rays are exceedingly feeble, on account of the long life of these bodies.

*From these remarks it will be clear that there is no essential difference between the orders of ranges of the lives of  $\beta$ -ray and  $\alpha$ -ray bodies.*

(b) Ellis<sup>4</sup> has shown in numerous papers that one  $\beta$ -particle is emitted per one disintegrating atom, so that the possibility that the expulsions are due to some external agency seems to be ruled out. They are spontaneous processes like  $\alpha$ -ray disintegration.

(c) The distribution of energy in the  $\beta$ -ray spectrum—This point has formed the subject of investigation by a large number of workers. The curves bear some resemblance to Planck's curve for blackbody radiation but unlike that curve, it has got a limit on the high energy side and the maximum is ill-defined. They also present some similarity to the curves obtained by Kuhlenskampf on the distribution of intensity in the continuous X-ray spectrum.

There has been an idea that the  $\beta$ -rays are probably emitted with quite a definite energy from the nucleus, but in its passage through the outer shell

of electron, it suffers diminution in energy owing to collision or scattering but this view has been disproved by Ellis.<sup>5</sup> Lately, attempts have been made to determine the maximum energy as accurately as possible and to deduce from it a relation similar to that of Geiger and Nutall for  $\alpha$ -ray bodies.

The latest exponent of this idea is Sargent<sup>6</sup> who found in a recent paper that every  $\beta$ -ray disintegrating atom is distinguished by having a definite end-point in its energy-spectrum. But a reference to his figures shown in Table VI, p. 670, and his curves on p. 671, Fig. 2, shows that there is not much evidence of a relation. For the points lie on three distinct curves, and the radioactive bodies belonging to the same family do not lie on the same curve. Secondly, if the Geiger-Nutall law for  $\alpha$ -ray bodies is expressed in the form  $\lambda = aE^n$  where  $E$  = energy of the  $\alpha$ -particle varying between 4 to 8 million electron-volts,  $\lambda$  varies from  $10^5 \text{ sec}^{-1}$  (Th C') to  $10^{-18} \text{ sec}^{-1}$  (U),  $n$  is found to vary from 65 to 100. But for the  $\beta$ -ray bodies,  $E$  varies from  $3.5 \cdot 10^4 \text{ evs}$  to  $3.15 \times 10^6 \text{ evs}$ , i.e., a range of about 1 to 100, but  $\lambda$  varies from  $10^{-2} \text{ sec}^{-1}$  to  $10^{-9} \text{ sec}^{-1}$  and if  $\lambda$  be put  $= bE^n$ ,  $n$  varies from 3 to 7. The attempt to trace a causal connection between the decay constant and the maximum  $\beta$ -ray energy does not appear to have been successful. We shall see later that no such causal connection is expected.

*The fact that the  $\beta$ -ray bodies follow the same law of decay as  $\alpha$ -ray bodies can, however, point to only one conclusion, i.e., the phenomenon is due to the leakage of  $\alpha$ -rays through a potential barrier, but somehow the  $\alpha$ -ray does not leave the nucleus, but a  $\nu$ -ray is generated in its place.*

Bohr weighs the probability that the continuous  $\beta$ -ray energy spectrum may be due to differences in the energy contents of the individual parent atoms leading to small and undetectable differences in their mass, but finally decides against this view. The following are his words.

"Unless the expulsion of  $\beta$ -rays from atomic nuclei, contrary to expectation, is not a spontaneous process but caused by some external agency, the application of the principle of energy conservation to  $\beta$ -ray disintegration would accordingly imply that the atoms of any given radio element would have different energy contents. Although the corresponding variations in mass would be far too small to be detected by the present experimental methods, such definite energy differences between the individual atoms would be very difficult to reconcile with other atomic properties. In the first place, we find no analogy to such variations in the domain of non-radioactive elements. In fact, as far as the investigations of nuclear statistics go, the nuclei of any type, which have the same charge and within the limits of experimental accuracy, the same mass, are found to obey definite statistics in the quantum mechanical sense, meaning that such nuclei are not to be regarded as approximately equal, but as essentially identical. This conclusion is the more important for our argument, because in absence of any theory of the intra-nuclear



electrons, the identity under consideration is in no way a consequence of quantum mechanics, like the identity of the extra-nuclear electronic configurations of all atoms of an element in a given stationary state, but represents a new fundamental feature of atomic stability. Secondly, no evidence of an energy variation of the kind in question can be found in the study of the stationary states of the radioactive nuclei involved in the emission of  $\sigma$  and  $\gamma$  rays from members of a radioactive family proceeding or following a  $\beta$ -ray product. *Finally, the definite rate of decay which is a common feature of  $\alpha$ - and  $\beta$ -ray disintegrations points even for a  $\beta$ -ray product, to an essential similarity of all the parent atoms, in spite of the variation of the energy liberated by the expulsion of the  $\beta$ -ray.* In absence of a general consistent theory embracing the relationship between the intrinsic stability of electrons and protons and the existence of the elementary quanta of electricity and action, it is very difficult to arrive at a definite conclusion in this matter."

We have quoted this passage in full, because after this paper was written, we came across a paper by Beck<sup>7</sup> where this idea of hypothetical differences in the energy contents of the individual parent atoms resulting in small and undetectable differences in their mass has been revived to account for the continuous energy distribution amongst the ejected  $\beta$ -rays.

Finally, in order to explain events, Bohr wants to sacrifice the law of conservation of energy and suggests the following process:

"At the present stage of atomic theory, however, we may say that we have no argument, either empirical or theoretical, for upholding the energy principle in the case of  $\beta$ -ray disintegrations, and are even led to complications and difficulties in trying to do so. Of course, a radical departure from this principle would imply strange consequences, in case such a process could be reversed. Indeed if, in a collision process, an electron could attach itself to a nucleus with loss of its mechanical individuality, and subsequently be recreated as a  $\beta$ -ray, we should find that the energy of this  $\beta$ -ray would generally differ from that of the original electron. Still just as the account of those aspects of atomic constitution essential for the explanation of the ordinary physical and chemical properties of matter implies a renunciation of the classical ideal of causality, the features of atomic stability, still deeper-lying, responsible for the existence and the properties of atomic nuclei, may force us to renounce the very idea of energy balance."

The above short summary will probably convey some idea regarding the complexity of the problem.

## 2. Electrofission of Light Quanta

It appears that the  $\beta$ -ray disintegration admits of a rather simple interpretation on the basis of the recent experiments by Anderson and Neddermeyer,<sup>8</sup> Meitner and Hupfeld,<sup>9</sup> Curie and Joliot<sup>10</sup> on the production of pairs

of positrons and electrons by impact of hard  $\gamma$ -rays with atomic nuclei. As the description of this fundamental discovery, which promises to throw a flood of light on nuclear physics, is still scattered over the pages of many scientific journals, we try to give a connected account of it here. Skobelzyn<sup>11</sup> was the first to use vertical Wilson Chambers placed within a horizontal magnetic field for photographing the track of cosmic rays. He found that the cosmic rays gave rise to tracks of  $\beta$ -rays possessing extremely high energy. In some cases, the mass-equivalent of the energy was as great as 50 ~ 100 times the rest-mass of the electron. On repeating these experiments, Anderson<sup>8</sup> found that in addition to the tracks due to high energy  $\beta$ -rays there were others possessing equal curvature, but bent in the opposite direction. From the nature of ionisation along these tracks, it was clear that they were due to particles of the same type as electrons, but possessing an opposite, *i.e.*, a plus charge. To this particle, which is the exact positive analogue of the electron, the name *positron* was given. Subsequently Meitner and Hupfeld<sup>9</sup> obtained similar paired tracks of electrons and positrons by taking Wilson photographs of Be-radiations impinging on Pb and Anderson and Neddermeyer and Curie and Joliot<sup>10</sup> showed that even the hard  $\gamma$ -rays from ThC' having the energy 2.0 *mevs* can give rise to such paired tracks (*mevs* stands for million electron volts).

### How are the pair positron and electron produced?

Anderson and Neddermeyer, and Blackett and Occhialini<sup>11</sup> further showed that this production of "paired ions" accounts for a number of unexplained facts. Gray and Tarrant<sup>12</sup> had previously shown that hard  $\gamma$ -rays show an anomalous absorption which is not accounted for by the Klein-Nishina formula for scattering. The anomalous absorption was found by them to start at the  $\gamma$ -ray energy 2 to 3 *mevs*. Later Gentner<sup>13</sup> fixed the limit at 1.2 *mevs*. We have to remember in this connection that  $m_0c^2$ , the rest energy of the electron corresponds to  $5 \times 10^5$  *ers* and thus the energy of a pair of electron and positron at rest is equivalent to 1 *mevs*. Hence there is a connection between the beginning of anomalous absorption, and the production of "paired ions." Blackett and Occhialini<sup>11</sup> suggested that within the nucleus, the  $\gamma$ -ray is split up, under the intense electric field, into a pair consisting of a positron and an electron. Oppenheimer and Plessett<sup>14</sup> regarded the phenomenon as a photo-electric effect, the  $\gamma$ -ray quantum lifting an electron occupying one of Dirac's negative energy states into a positive energy state, thus simultaneously creating an ordinary electron and a "hole" which according to the ideas of Dirac will correspond to the positron (*vide* § 3). Curie and Joliot<sup>10</sup> have proposed to denote this phenomenon as "*materialisation of quanta*."

Blackett further showed that the hypothesis of the splitting of the quantum inside the nucleus explains another interesting observation by Gray<sup>12</sup> and others. The former has subjected the nuclei of many atoms to hard

$\gamma$ -rays from  $\text{ThC}'$  and found that the nuclei were thereby excited to a fluorescent radiation of approximate wavelengths 12X. units and 24X. units. The first possesses an energy of 1 *mevs* and the second  $\frac{1}{2}$  *mevs*. According to Blackett, though the  $\gamma$ -ray may split up inside the nucleus into a pair of positrons and electrons, but the two may again combine either inside the nucleus or just outside. When they combine inside the nucleus only one quantum of energy 1 *mevs* units may be produced. If they combine outside, two quanta each of energy 0.5 *mevs* units will be produced.

We are of opinion that the phenomenon of conversion of a  $\gamma$ -ray into a pair of ions of opposite sign, confirmed by so many investigators in different parts of the world, should be designated by a more expressive term than Curie and Joliot's *Materialisation of Quanta* and the round-about phraseology about holes, etc., borrowed from Dirac's theory should be avoided, and we have ventured to suggest the term '*Electrofission of Light Quantum*...' which clearly expresses the idea that under the influence of the nuclear fields, the quantum of energy undergoes a '*fission*' into elementary charges of opposite sign, the balance of energy being distributed as kinetic energy amongst the two products in a way which is still to be determined. The possibility of the reverse process of two charges neutralising each other in a direct collision has been postulated by many astrophysicists in a slightly different form. But when these predictions were made, the positive unit of electricity was known to be always associated with the mass in a proton, and nobody could conceive of a positron, hence they always talked of annihilation of proton and electron, but the hypothesis has always lacked vigour on account of want of experimental proof. The process as now actually found is different from the early hypothesis about annihilation in many other points.

### Theoretical Predictions about Positron

It may be added at this stage that grounds for the advent of the positron were to some extent prepared by the predictions of Dirac<sup>15</sup> from his relativistic theory of the electron. In this he was first led to postulate the existence of an elementary particle having the charge- $e$  but possessing the negative energy  $-mc^2$ . Such a particle (the anti-electron) would possess very weird properties which have not been observed. We quote from Gamow.

"For such particles the force and acceleration are directed in opposite directions. If two electrons, one of 'positive' and the other of 'negative' mass, meet then the first will be repelled and the second attracted to the other one; both electrons will fly away one behind the other with infinitely increasing velocity, giving an amusing picture of electronic races."

Later, Dirac developed a theory of 'holes' to account for 'positive charges'. He postulated that in Nature all the negative energy states are usually fully occupied, but sometimes a hole may appear. A positive energy

electron will then jump into the hole, resulting in the neutralisation of charges and release of the energy  $> 2 m_0 c^2$  as radiation in the form of one or two quanta. The process is thus equivalent to the so-called annihilation of charges. The 'hole' can be identified as a "unit positive charge". But it could not be identified with the 'proton' because the mass of the proton is 1836 times heavier than that of the 'hole'. The discovery of the positron exactly corresponds to Dirac's hole, but sweeps away the misleading ideas about particles capable of possessing "negative energy-state". These ideas are not a little responsible for creating confusion in contemporary scientific thought. Instead of an anti-electron with a negative energy we have now a straightforward positive analogue to the electron with positive charge and positive energy.

### The Proton

The question of the nature of the Proton now becomes a problem. According to one view, the proton is not a fundamental particle but is a compound of the neutron and the positron. If this view be correct, the neutron is merely 'mass' possessing an inherent tendency to capture positrons, but behaving in a different way towards electrons which they cannot capture for if this could take place, we could obtain a negative proton. There is also certain amount of experimental evidence in favour of this view. Anderson and Neddermeyer, as well as Curie and Joliot found in their experiments on *Electrofission* of  $\text{ThC}'$   $\gamma$ -ray quantum that more electrons are obtained than positrons, Curie and Joliot<sup>10</sup> give the following figures.

*Number of positrons per 100 electrons (Magnetic field 1100 gauss).*

Al	Cu	Pb	U
5	18	30	40

But working with cosmic rays which can now be definitely taken to be super  $\gamma$ -rays, it has been found by Anderson as well as Kunze<sup>16</sup> that the number of positron tracks is equal to the number of electron tracks. These results, can be explained on the hypothesis that positrons are easily absorbed by the constituents of the nucleus, possibly neutrons, while electrons are repelled by them. Only very high energy positrons can resist capture by nuclei. Further, if the neutron, the electron and the positron are fundamental particles, they should possess the angular momentum  $\frac{1}{2} \frac{h}{2\pi}$  (and be guided by Fermi-Statistics). The protons according to this view may have varying angular momentum depending upon the state of the combination between the neutron and the positron, a view which seems to be in agreement with the latest results of

Stern and Eastermann.<sup>17</sup> According to Chadwick,<sup>18</sup> however, the proton is probably fundamental, and the neutron is a "dipole" composed of the proton and the electron. As the difference of mass on the two views is of the order of '00054, the question cannot probably be ever determined by a precision estimation of masses, but only by investigation of the response of the neutron to light quanta. For, Chadwick's neutron being a dipole, would be highly reactive towards electromagnetic radiation, while the mere 'mass-neutron' is not expected to be reactive. Even on this point, we are not on very sure grounds, for according to one of us, the neutron is a magnetic dipole, composed of two free Dirac's magnetic poles separated by a distance of  $\frac{e^2}{Mc^2}$  which is  $\frac{2}{3}$  times the protonic radius, but these views have no effect on the present course of investigation.

Though not directly connected with the subject-matter of this article, it may be pointed out that the two views regarding the proton will have different consequences in astrophysics. According to many astrophysicists, hydrogen is found in abundance in many stars, and there is a likelihood that the chief constituent of all stellar matter is hydrogen. This must exist in the interior as protons. The proton, if it is a compound will be further broken up into the neutron and the positron, for the binding energy is small, between  $10^4$  to  $10^5$  evs, and even the smallest temperature ascribed to stellar interiors is sufficient for the complete breaking of the proton. The other (Chadwick's) view does not allow this breaking, for the proton being fundamental cannot be further subdivided. So on the first view the stellar core will consist of neutrons, positrons and electrons; while on the second view, it will consist of protons and electrons. This is fraught with far-reaching consequences. For the neutrons have been found to possess the remarkable property of passing through matter till they are stopped by the nucleus, and when they strike the nucleus, they excite radical changes in it, resulting in the emission of protons,  $\gamma$ -rays.  $\alpha$ -particles. One of us is at present engaged in working out a model of a star whose interior is mainly composed of neutrons.

### Annihilation of Charges

In this connection, we may refer to the hypothesis about annihilation of matter advocated by Jeans and Eddington<sup>19</sup> to account for the source of stellar energy. A certain amount of vagueness is always attached to such hypothesis, for annihilation literally means to be reduced to nothing, but the process described here is very different from unalloyed nihilism on the part of fundamental particles, for when an electron and proton hit each other, a neutron and a  $\gamma$ -ray is produced. There is no violation of the principle of

conservation of energy or momentum, so nothing is annihilated except that the charges seemingly disappear. The energy of the  $\gamma$ -ray is available for supplying the stellar energy, but it is not yet known whether the mass of the neutron can be converted into energy. Again, when an electron and positron collide outside a nucleus an application of the principle of conservation of energy and linear momentum shows that two quanta must be produced in their place. If they collide inside the nucleus, there may be one quantum as the nucleus can bear certain amount of the shock and thus ensure the obedience to the law of conservation of momentum. In both these processes, there are more variables than equations, and hence the energy of the quanta cannot be uniquely determined. In none of these processes of collision there is either annihilation of mass, or energy, and not even of charges, for in the quantum formed, the two charges probably retain their individual existence as components of a dipole moving with the velocity of light, and they can again be separated when a "fission" takes place. This picture is very different from what is conveyed by Eddington's picturesque description of the phenomenon as a "joint suicide of the electron and the proton."

### 3. Explanation or $\beta$ -Ray Activity

We shall now discuss how the  $\beta$ -ray activity can be explained. It is clear that if a  $\gamma$ -ray or supergamma (cosmic) ray coming from outside can split up inside the nucleus into an electron and a positron, it will be much more easier for a  $\gamma$ -ray, of sufficient energy, which is produced within the nucleus to undergo spontaneously such a process of electrofission. Of the pair produced, the electron will be ejected as a  $\beta$ -ray, but the positron cannot usually escape, for it will be prevented by the potential barrier from escaping when such barriers exist, or attach itself to some neutron which is present inside the nucleus. For we have already seen that the neutron has an affinity for the positron, but none for the electron. The net charge in any case will be increased by unity, as is observed in  $\beta$ -ray disintegration. It attaches itself to a neutron,  $\gamma$ -rays of small energy of the order of .05 mevs would probably be given off, which are always observed in a  $\beta$ -ray disintegration. It is not difficult to account for the continuous distribution of  $\beta$ -ray energy, for the primary  $\gamma$ -ray while undergoing '*Internal electrofission*' may have its energy divided between the pairs within wide limits and a certain amount of energy will be communicated to the nucleus. But exact mathematical calculations can be carried out only when more data are forthcoming. The problem of annihilation of two charges of opposite sign which is the converse of the present problem has been discussed by Dirac<sup>20</sup> Tamm, and Oppenheimer on the basis of Dirac's holes as positrons.

According to the above view, the  $\beta$ -ray emission is only a secondary process, the primary phenomenon which starts the chain of events which we call a  $\beta$ -ray disintegration is the generation of primary  $\gamma$ -ray within the nucleus. We may now ask ourselves: how is this  $\gamma$ -ray generated? For this, a discussion of the recent theories of a  $\alpha$ -ray disintegration is necessary.

It is now well known that classical mechanics offered no solution to the problems of radioactivity. Gamow, and Gurney and Condon first suggested methods for explaining many features of radioactivity from the standpoint of wave-mechanics. The methods were elaborated in great detail by Gamow who succeeded in achieving a good deal of success in explaining the essential features of  $\alpha$ -ray disintegration and  $\gamma$ -ray origins. Very substantial contributions were also made by Laue, Fowler, Fowler and Wilson, Atkinson and Houtermans, Schrodinger and others.<sup>21</sup>

All these works suffer from the defect that we have as yet no sure knowledge of the structure of nucleus, *i.e.*, of the constituent particles, the statistics obeyed by them and the laws of interaction towards each other. Hence, as in the earlier stages of study of many other branches of science, ad hoc hypotheses based on previous knowledge, have to be invented, and the value of these hypotheses is determined by the amount of success achieved by them. It now seems to be fairly certain as mentioned in the introduction that the nucleus consists of protons and neutrons only, and that there are no free electrons (or negative charge in any form) in the nucleus. Most of the protons are combined in the form of  $\alpha$ -particles. From a scrutiny of Aston's mass-defect curves it has been deduced that elements after Pb are mostly built up by the addition of only  $\alpha$ -particles to the Pb nucleus. Thus U (238/92) the parent of radioactive elements having  $A=4n+2$  consists of a Pb nucleus (206/82) with 8  $\alpha$ -particles about it. Th (232/92) the parent of radioactive elements having  $A=4n$  consists of the lead nucleus (208/82) with 6  $\alpha$ -particles about it. The mass-defect curve shows that the binding force of these  $\alpha$ -particles is very small, *i.e.*, they can be regarded as free to a certain extent. They are prevented from leaving the nucleus by the existence of a potential barrier about the nucleus, whose height is larger than the energy of the  $\alpha$ -particles in the crater. According to classical mechanics it will be impossible for the particles to leave the nucleus, but it was suggested by Gamow, and Gurney and Condon that according to wave-mechanics they can be regarded as waves, and thus possess the property of leaking through the barrier. The rate of leakage through the barrier determines the decay of the elements. Various hypotheses have been postulated regarding the height, size and form of the barrier, but the final results agree in their essential features. There is, however, a large amount of divergence in the methods of mathematisation of the ideas. Laue and others take simplified cases,<sup>21</sup> in which the process is regarded as stationary

and calculate the rate of leakage through an oblong-shaped potential barrier. Though the mathematics is much simplified, the picture does not evidently correspond to facts as the process cannot be regarded as stationary (independent of time). Gamow,<sup>22</sup> on the other hand, introduces complex eigen-values, and by a suitable formulation of boundary conditions, obtains values of decay constants as well as of the eigen-values for the energy of the  $\alpha$ -particles inside the crater. His final results are

$$\log \lambda = \log \frac{h}{4\pi m r_0^2} - \frac{8\pi^2 e^2 (Z-2)}{h V_e M} + \frac{16\pi e m^{\frac{1}{2}} (Z-2)^{\frac{1}{2}}}{h M} r_0^{\frac{1}{2}}$$

$$E = \frac{n^2 h^2}{8\pi m r^2} + U_0 = \frac{1}{2} m V_e^2$$

where  $e$ ,  $h$  and  $Z$  have their usual meaning.  $M$  is the mass of the  $\alpha$ -particle and  $V_e$  is the velocity with which it escapes.  $r_0$  is the "radius" of the product nucleus and  $V_0$  mean potential energy of an  $\alpha$ -particle inside it.

It is seen from the above formula that they involve two constants, *viz.*,  $r_0$  the equivalent radius of the crater, and  $V_e$  the velocity of ejection of the  $\alpha$ -particle. According to our picture,  $r_0$  should not much vary for elements belonging to the same radioactive family while the radius  $r_0$  is found to vary in a regular way from U to RaC and from Th to Th A. We get abnormally low values for it when we come to those interesting products RaC, ThC and AcC which disintegrate in a dual fashion, emitting both  $\alpha$ - and  $\beta$ -rays. The value of  $r_0$  falls from  $8.3 \times 10^{-12}$  for RaA to  $6.3 \times 10^{-12}$  for RaC; and from  $8.1 \times 10^{-12}$  for ThA to  $6.6 \times 10^{-12}$  for ThC.

We revert again to the question as to how the primary  $\gamma$ -ray referred to above which, by undergoing internal electrofission gives rise to the observed  $\beta$ -decay, is generated. It is reasonable to postulate that there are more than one potential barrier inside a nucleus, though their exact nature (*i.e.*, their height and width) and forms can only be determined when we have a sufficient knowledge of the structural arrangement of the particles constituting the nucleus. Our assumption is that the primary  $\gamma$ -ray is generated by the leakage of an  $\alpha$ -particle through an internal potential barrier, *i.e.*, the  $\alpha$ -particle leaks from one crater to another, both within the nucleus. It occupies a lower energy level in the new crater and the balance of energy constitutes the primary  $\gamma$ -ray. This primary  $\gamma$ -ray suffers an electrofission producing a positive and a negative electron. The positive electron attaches itself to one of the neutrons present inside the nucleus, thus raising the nuclear charge by unity. The negative electron is ejected, which constitutes the usual  $\beta$ -ray. The combination of the positron with the neutron will liberate some energy (nearly equal to the difference between the masses of positron+neutron, and the proton) and this may account for the soft  $\gamma$ -rays that usually accompany a



$\beta$ -disintegration. The life of the  $\beta$ -decay is determined by the rate of leakage of the  $\alpha$ -particle from one inside crater to another and hence to the first order will be independent of the energy of the  $\beta$ -rays. Thus no simple relation (unlike the case of  $\alpha$ -decay) is expected to exist between the maximum energy of  $\beta$ -rays and the life of  $\beta$ -decay, a conclusion which is more or less borne out by Sargent's curves.

On the above view it is to be expected that occasionally a positron may not be captured by the neutron, and it may emerge. The presence of positrons associated with the natural  $\beta$ -decay as suggested by Skobelzyn's experiments lends support to the views herein stated.

The explanation of the continuous energy distribution in the  $\beta$ -ray spectrum offers no special difficulties. In our case the energy of the primary  $\gamma$  ray is shared between the positron and the electron, and so the energy of the electron can vary from zero to a maximum ( $h\nu = c + 2m_0c^2$ ). The exact form of the distribution curve can only be calculated when we make additional assumptions regarding the mechanism of interaction. This will be examined on a future occasion.

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# ON THE DETERMINATION OF THE VALUES OF $\gamma$ FOR AIR SATURATED WITH WATER VAPOUR AT VARIOUS TEMPERATURES.

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## Introduction

The value of  $\gamma$  is very important from the thermodynamical point of view, and a precise study of its variation has an important bearing on the molecular structure of gases and is of great help towards a better understanding of their dissociating equilibria. But the available data of high precision on  $\gamma$  is poor excepting for a limited number of gases and organic vapours. There is hardly any data for  $\gamma$  for air which is completely saturated with water vapour. In the present paper the author wants to report some results of his experiments on the determination of  $\gamma$  for air saturated with water vapour which were carried out at various temperatures ranging between about 15°C. and 80°C. The method used was to determine accurately the velocity of sound in saturated air at various temperatures by means of a resonating tube. This was done by setting up stationary waves in the tube by means of a telephone diaphragm, actuated by a source of constant frequency, *viz.*, a valve-maintained tuning fork oscillator, and measuring the internodal distance  $\lambda/2$ . The tube-velocity of sound in saturated air at any particular temperature was then obtained as a product of the wavelength,  $\lambda$ , and the frequency 'N' of the source. We can then obtain the value of  $\gamma$ , from the basic formula  $V = \sqrt{\frac{P \cdot \gamma}{\rho}}$  . . . . (1) if the values of P and  $\rho$  be known.

Recently C. D. Reid<sup>1</sup> has studied the increase in the velocity of sound due to the presence of moisture in air. He determined the velocity of sound at 20°C. in dry air, humid air (45% relative humidity) and in air completely saturated with water vapour (100% relative humidity) and by plotting this increase in the velocity of sound against the percentage relative humidity he has obtained a straight line graph satisfying the empirical relation,  $V_H = V_0 + 0.14 H$  . . . (2) where  $V_H$  is the velocity at any relative humidity at 20°C.;  $V_0$  is the velocity in dry air at 20°C. and H is the relative humidity. The author has also plotted the experimental values for a wide range of

temperature, of  $(V_H - V_O)$ , the excess of the velocity of sound in saturated air over the velocity of sound in dry air at the same temperature, against the moisture content as well as against the temperature. These curves may be seen under section XIII.

## II

### Previous Experimental Methods and Discussion

The method of determining the velocity of sound in gases in resonating tubes is still considered by some to be not free from uncertain errors due to the tube-effects such as the dependence of tube-velocity, on the frequency, on the roughness of the wall surface, on its thickness and on the nature of the material of the tube, etc. But through the efforts of many observers in the recent time, specially G. W. C. Kaye<sup>2</sup> and G. G. Sherratt, who made precision measurements of the velocity of sound in different gases, contained in resonating tubes of different diameters and material, many of the uncertain tube-corrections have been totally removed, and for all practical purposes the validity of the Helmholtz-Kirchhoff expression has been firmly established. The divergence of results in connection with the tube-correction is mostly due to inaccurate and inconsistent experiments for which the theory cannot be blamed. This point is discussed fully later on.

On the other hand, the method of determining the velocity of sound in gases depending upon what is sometimes called Pierce's acoustical interferometer, making use of ultrasonic waves (generated either by the quartz crystal oscillator or by the magnetostriction oscillator) is free from all difficulties of tube-correction, on account of very high frequencies used, but involves other uncertain and irregular effects arising out of the diffraction phenomena (and impedance effect) which cause the velocity of sound to be higher than the accepted free-space value; the effect being considerable in case the measurements of wavelength are made when the source and the reflector are not very far from each other or when the frequency used is near about 42 kilocycles<sup>3</sup> per second. Further, the smallness of wavelengths measured (for instance at 60,500 cycles per second, the wavelength in air is only about 0.57 cm.) demand an extreme sharpness of maxima (or minima). But such sharpness of maxima, in practice, cannot be attained for obvious reasons that the positions of the maxima are ascertained from the deflection of the micro-ammeter needle, which even in the vicinity of a reaction, are not very steep. This point, however, may be illustrated from the careful experiments of Martin Grabau<sup>4</sup> who states that the accuracy of the setting of the mean position of a maximum is about 0.5 mm. for a frequency 19,790 cycles per

second (wavelength about 1.75 cms). Thus the ratio of the accuracy of setting to the wavelength observed is  $\frac{0.5}{17.5}$  which is  $\frac{1}{35}$ . While in the present experiments (with the resonating tube) to be described, the accuracy of the setting of the mean position of a maximum was within 0.5 mm. for a wavelength about 350 mms. and hence the ratio of the accuracy of setting to the wavelength is  $\frac{1}{700}$ . This may further be stressed by quoting the divergence of results which W. H. Pielemeier,<sup>5</sup> working carefully with a Pierce acoustical interferometer, obtained in the case of dry air. His values of the velocity of sound in dry air at 0°C. are as follows: 333.3 m. per sec., 332.8 m. per sec., 334.5 m. per sec., 333.8 m. per sec. and 333.7 m. per sec., which differ from each other to such an extent as to be practically of little use if one wishes to calculate the precise value of  $\gamma$  for dry air. These considerations\* led the author to adopt the resonating tube-method for determining the internodal distances with a constant frequency source of sound, and it will be sufficiently proved in subsequent sections of this paper that it is possible to obtain the accurate values of the velocity of sound by the above method.

### III

#### Description of the Apparatus

The apparatus consisted mainly of a movable source of sound mounted at an end of a brass rod which could move it throughout the entire length of the resonating tube; and a fixed reflector. No listening side-tube† was used but instead a microphone was placed just beyond the fixed reflector which was a thin disc of mica. A section of the main portion of the apparatus is shown in Fig. 1. AB is a straight pyrex glass tube (specially ordered for

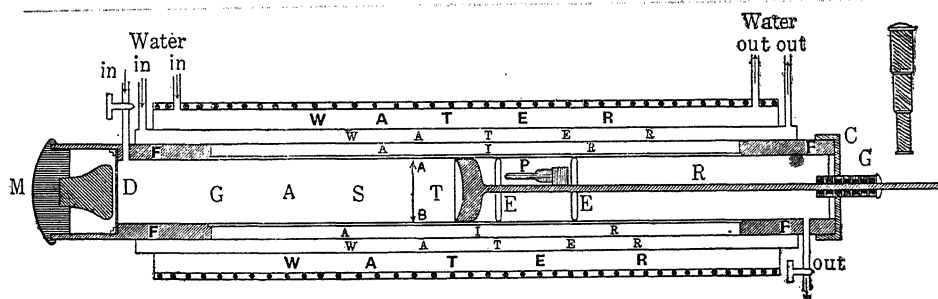


Fig. 1

these experiments) which is about 170 cms. long and 2 mms. thick with a nearly uniform bore of about 6.08 cms. diameter. FF are the brass flanges

\* Moreover, it is very difficult to maintain the oscillations of the quartz crystal in the presence of the large quantities of moisture contained in the saturated air and hence the accuracy of results is impaired.

† See discussion by Mr. D. A. Oliver, Proc. Phys. Soc., Vol. 43; 252; 1931.

fixed at each end of the resonating tube AB. Each flange in turn carries a side-tube of about 3 mms. diameter to serve as an inlet and an outlet for the gas under observation. D is a mica disc about 0.2 mm. thick which is mounted perpendicular to the axis of the tube by means of an arrangement of threaded brass rings which keep the mica disc rigidly fixed. The mica membrane D thus closes one end of the resonating tube and, as noticed before, serves the purpose of a reflector. This end of the tube, 'the reflector end,' to call it so, was made air-tight by means of plaster of paris. The other end of the resonating tube, the telephone end, is closed by means of a brass cap C at the extreme mouth of the flange F. In the centre of this cap there is an air-tight annular gland through which the brass rod R can be moved to and fro without any fear of leakage. The construction of the annular gland G is shown in Fig. 1 which is more or less like the flange arrangement used in piezometers of the usual type. The brass cap C at the end of the tube is also fitted with six terminals insulated from each other by means of mica and ebonite washers. Plaster of paris was used to make this end of the tube also air-tight, as far as possible. A telephone having a resistance of about 2000 ohms, and of such a size as to fit in the tube correctly, was mounted on the brass rod R so rigidly that there was no danger of any relative motion between the rod and the telephone diaphragm. Particular care was taken to keep the telephone diaphragm, perpendicular to the axis of the tube so that the reflector and the diaphragm of the telephone were always parallel to each other. In order to completely avoid the annular space left between the rim of the moving telephone and the wall of the tube, we wrapped round the rim a thin strip of flannel which acted like an elastic soft pad, so that this piston was a good fit in the resonating tube. The pad also served the purpose of eliminating all the undesirable creaking noises produced by the metallic telephone rim of the tube. To allow a more or less free passage of air past the rim of the telephone three small grooves were cut in the pad round the rim. Behind the telephone a perforated ebonite disc E of almost the same diameter as the telephone was mounted on the brass rod, which passed through its centre so rigidly that it moved along with the telephone throughout the tube. Immediately behind the ebonite disc E a platinum thermometer of length about 18 cms. and weighing about 105 grams was mounted by means of brass screws on the brass rod itself, and about 3.5 cms. away from the head of the platinum thermometer another perforated ebonite disc E was fastened rigidly on to the brass rod. Just beyond the mica reflector end of the resonating tube a small Lapel<sup>6</sup> microphone which was mounted on a circular wooden bed was fitted in such a way that the face of the microphone was about a centimetre away from the mica membrane. The circular wooden bed of the microphone fitted tightly in the brass flange F and thus kept the microphone fixed at that particular position. To match with this microphone

an audio-frequency transformer was used. Any sound received through the microphone was directly heard in the head-phones which were connected to the secondary of the transformer. The apparatus was placed on a V-shaped wooden platform.

#### IV

##### Sound Production and Remarks on the Constancy of the Frequency of the Source

The source of sound used in these experiments for setting up stationary waves inside the resonating tube was the telephone T electrically connected to the output terminals of a valve-maintained tuning fork oscillator such as that described by D. Dye.<sup>7</sup> The tuning fork used in the oscillator was made of 'elinvar' steel (Valentine and Carr) with a nominal frequency of about 1000 cycles per second. It was mounted very solidly in a solid block of brass which in turn was very rigidly riveted to a solid rectangular brass plate and the whole arrangement with the electrical attachments was placed in a small wooden box having a thick layer of soft woollen pad at its bottom. The same valve (Phillips A. 415) was used throughout all these experiments.

After making some preliminary experiments with sounds of different intensities in the resonating tube, we found it best to keep the anode voltage at 60 volts; the anode current in this case being of the order of 1.2 milliamperes. Voltages higher than this were deliberately not used in order to avoid sound waves of great intensity in the tube. To maintain the frequency constant, as far as possible, the oscillator was left completely undisturbed so that there was no danger of any change of frequency due to the causes, namely—(1) variation of filament current and anode voltage, (2) variation of grid and anode condensers, (3) variation of the polarising magnetic field, (4) variation of energy taken from the output winding, and (5) variation due to adding a mass to various parts of the mounting or to tilting of the fork.

The only cause of variation of frequency was the temperature of room which varied roughly between 28° C. and 36° C. But since the tuning-fork used is made of elinvar steel for which the temperature coefficient of frequency change is known to be very small, the change in the frequency for a range 28° C. to 36° C. may amount only to about 0.8 parts in ten thousands which being well within the experimental error was completely neglected. The frequency of the note under the actual conditions of the experiments (velocity experiments) as heard in the head phones was determined by comparing this with a certified tuning-fork also of elinvar steel (Valentine and Carr) having a frequency 1000 cycles per second when mounted solidly, as directed in the certificate. Beats were counted and the time was taken by means of a stop watch. This part of the experiment was extremely difficult because of the

fact that the beats counted were very nearly six per second and hence extreme care had to be taken for accurate counting. However, many independent readings by different persons were taken to exactly fix the number of beats per second and the results of all such readings were found to be between 5.9 and 6.0 beats per second, so that the frequency of the note in the resonating tube under identical conditions of the actual velocity experiments comes to be  $(1000-6) = 994$  cycles per second. To test this point further and to facilitate the counting of beats accurately enough for our experimental purposes we loaded the prongs of the certified tuning-fork with equal quantities of soft wax placed symmetrically on both the prongs. The quantities of wax were at first so adjusted that absolutely no beat was observed. After this the quantities of wax on the prongs were halved and the beats produced under this condition were carefully counted. The mean of several such readings, for one experiment, was found to be 3.02 beats per second. Assuming a linear variation of beats with the added masses (wax) to the prongs (a fact approximately true for such a short range as this) we could conclude that the frequency of note in the resonating tube was  $(1000-3 \times 2) = 994$  cycles per second, with a probable error of 0.1 per cent. It may be remarked here that the response of the ear to various frequencies is not the same. It is maximum near 1024—1028 cycles per second so that the choice of this frequency 994 was of great value in obtaining a high degree of accuracy in locating the points of maximum intensity of sound in the resonating tube. Another advantage in the selection of this frequency 994 is that the wavelength (in air about 35 cms.) is about 12 times the radius of the resonating tube (3.04 cms). Lord Rayleigh<sup>8</sup> pointed out that the waves set up in a cylindrical tube will ultimately become plane, provided the ratio of wavelength to the radius of the tube is greater than about 3.4. Sherratt and Awbery<sup>9</sup> have also experimentally found out this ratio to be 4.5 which is a little higher than the theoretical one (3.4). This ratio 12 in our experiments is thus far higher than even the experimental value 4.5 and hence all possibilities of the sound pattern in the resonating tube getting complicated by virtue of its natural transverse vibrations are eliminated so that there cannot be introduced any uncertain error due to this alone in the measurement of the internodal distances.

## V

### Temperature Control and Measurement

Since the temperature coefficient of change in the velocity of sound in air is about 0.6 metres per second, *i.e.*, it is of the order of 1 in 600, it is necessary that the temperature should be controlled and measured with an error not exceeding about 0.5°C. in order to attain a probable accuracy

of about 1 in 1000 in the measurement of the velocity of sound. Naturally in the construction of the apparatus we paid a great attention to those parts of the apparatus which controlled and measured the temperature. To achieve this, a double jacketed cylindrical vessel surrounding the entire length of the tube as shown in figure 1, was constructed. This vessel then was wrapped up in asbestos sheets and nichrome wire was wound round it to heat it electrically. In winding the wire care was taken to keep the spacing as far uniform as possible except at both the ends where the wire turns were a little denser than at the central portion of the vessel. The vessel was again wrapped up in thick sheets of asbestos so that the wire turns lay in between parallel layers of asbestos sheets. A set of variable resistances and an ammeter were placed in series to vary the current in the furnace according to need. In this arrangement the heating or cooling of the gas enclosed in the resonating tube was effected very gradually. This arrangement thus ensured uniformity and accurate regulation of temperature throughout the whole duration of the experiment (about three hours). But we had to wait long before the temperature became steady, in fact we had to wait for eight to ten hours; but once it became steady the outside fluctuation of temperature had very little effect on it.

Still there were three regions in the apparatus which lost heat through radiation and conduction. These were (1) the microphone end of the tube and a little portion, within two cms., of the brass flange which was not enclosed in the heating vessel, (2) the end G of the tube could not be enclosed in the heating vessel, and (3) the brass rod itself which, of necessity must make to and fro motion while the wavelength measurements were being made. The first two sources of heat loss were partly stopped by wrapping heavily the uncovered portions with cotton-wool (not shown in Fig. 1). The microphone and its wooden frame served very well to stop the heat flow through that end. The third source of heat loss for practical purposes was not serious as may be ascertained from the readings in table (1). Temperatures lower than the room temperature were obtained and maintained constant by passing a slow current of cold water through the two jackets of the surrounding vessel. The supply of this cold water was obtained from a small cistern kept about five feet higher than the level of the vessel. We made attempts to keep the temperature of the flowing water constant during the experiment, but the arrangement was not so satisfactory as in the case of heating. Temperatures were measured by means of a platinum resistance thermometer. Pure platinum wire of 0.15 mm. diameter was used. The wires were welded by electricity. Resistances were measured on a calibrated Callendar and Griffith's bridge. The sensitivity of the galvanometer used was  $10^{-8}$  amperes per mm. deflection at a distance of one metre. Preliminary experiments were performed to determine the value of  $\delta$  for the particular wire used.



The determination of the sulphur boiling point was however not very accurate. The mean value of  $\delta$  found was 1.53. All precautions were taken to determine the fundamental interval accurately. The temperature with this arrangement could thus be ascertained accurately to about  $0.05^\circ\text{C}$ . To check the thermometer readings, we calibrated the platinum thermometer by directly comparing it with an accurate mercury thermometer reading up to  $\frac{1}{10}$  of a degree C.

While taking observations for the wavelength determination, the readings of the platinum thermometer also were taken at each maximum. A typical set of readings is given below :—

*Table 1*  
Room temperature  $32^\circ\text{C}$ .

Order of maxima.		Resistance of pt. ther. in ohms.	Temp. in degrees C.
1st max.	...	3.0702	51.4
2nd "	...	3.0703	51.4
3rd "	...	3.0704	51.4
4th "	...	3.0703	51.4
5th "	...	3.0704	51.4

## VI

### The Measurement of the Distances between Successive Maxima

For the precise measurement of half wavelength it is clear that the distance between the successive maxima should be very accurately measured. An error of  $\pm 0.05$  cms. for  $N=1000$ , (half wavelength about 18 cms.) may vitiate the result by as much as 1 metre in the velocity and much more in the cases of higher frequencies. In order to avoid this source of error we measured the length by the comparator method making use of a travelling microscope which was very rigidly fixed on the same wooden V-bench on which the main apparatus was fixed. Great care was taken to avoid any relative motion between the microscope and the resonating tube by clamping both the tube and the microscope very rigidly. The microscope was clamped in such a position that it could be focussed on the brass rod very easily. Since the rod moved through grooves on wooden supports it always remained perfectly horizontal and so always in sharp focus for all positions of the travelling microscope. Very fine cross ( $\times$ ) scratches at intervals of about  $10^6$  cms. were made on the entire length of the brass rod in

such a way that they were all in a straight line on it. The actual distances between the scratches were measured by the same travelling microscope. They are set down in table (2) below:—

*Table 2*  
Room temperature 32°C.

Distances between scratches on rod.			Cms.	Distances between scratches on rod.			Cms.
1st and 2nd	...	...	11.06	7th and 8th	...	...	10.02
2nd and 3rd	...	...	10.03	8th and 9th	...	...	10.07
3rd and 4th	...	...	10.05	9th and 10th	...	...	10.00
4th and 5th	...	...	10.09	10th and 11th	...	...	10.02
5th and 6th	...	...	10.01	11th and 12th	...	...	10.03
6th and 7th	...	...	10.00				

The temperature coefficient of the linear expansion for brass is about  $18.9 \times 10^{-6}$  cms. and so the increase in length per degree between any two scratches (10 cms. length) would be  $18.9 \times 10^{-5}$  cms. Since that portion of the brass rod which was under the microscope was exposed to the outside atmosphere it was comparatively cooler than the resonating tube and hence its temperature did not much differ from that of the room. Therefore the expansion in length between any two scratches on the rod would, in an extreme case, amount to only about  $4 \times 10^{-3}$  cms. and hence it was completely neglected. The effect of the sagging of the brass rod was also found to be quite negligible. This was studied by direct measurement, by placing a calibrated steel scale lengthwise on the glass tube, (the furnace having been removed) and taking readings on the travelling microscope scale as well as on the steel scale simultaneously. The scale of the travelling microscope was calibrated by comparing it with a standard platinum-iridium scale kept for this purpose in the laboratory. The procedure of the measurement of the half wavelengths was as follows:—

A node was located by a proper adjustment of the position of the telephone inside the resonating tube and the microscope moved to one of the convenient scratches on the brass rod, which lay in the range of the travelling microscope scale and the scale reading was noted down, say, the second scratch on the brass rod coincided with the cross wires of the microscope when its position on the scale was 4.03 cms. Then the rod R was slowly pulled out and the second node was located. The microscope was then moved to some other convenient scratch and the scale reading taken, say, the third scratch coincides with the microscope reading 11.56 cms. From these readings we immediately get the distance between the first node and the second node as  $(11.56 - 4.03) + 10.03$ ,

the distance between the second and the third scratch on the rod (table 2), which is equal to 17.56 cms. The same process was carried out for the rest of the nodes.

## VII

### The Location of Maxima

The points of maximum sound intensity, that is the nodes, being quite sharp, were easily detected. This was done, as follows, in two ways:—

(1) The brass rod carrying the telephone was moved slowly to a point of maximum intensity of sound which was audible only for a short range of

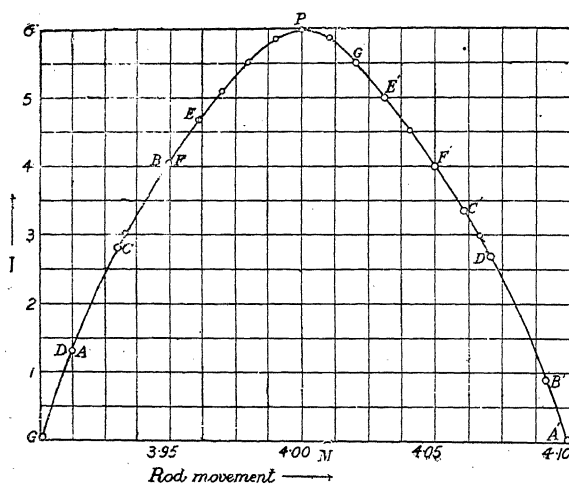


Fig. 2

the movement of the rod R (about 0.5 cm.) and two points were carefully marked, one while approaching the maximum and the other while receding from it, and the corresponding readings for these two points were taken on the travelling microscope scale. The mean of these two readings was then taken to indicate the correct position of the maximum on the assumption that the curve (Fig. 2), showing in an arbitrary way, the variation

of intensity with distances of the rod movement, is symmetrical with respect to the perpendicular P.M. The difference between these two readings was seldom greater than about 1.5 mms. and was usually of the order of 1 mm. Several such pairs of readings were taken to locate a maximum. This process was repeated for every maximum. The whole process was then repeated by moving the rod in the opposite direction and the readings were obtained. For brevity this complete process may be called a run. To ascertain the consistency in the exact position of the maxima, several such runs were made, and the mean of all taken to calculate the final results.

These experiments were mostly carried out in perfect silence during the night with all the doors of the room closed. This not only facilitated the location of maxima, but also helped a good deal in keeping the temperature constant to a better degree than in the day-time when the air draughts disturbed it slightly.

(2) The second method of locating maxima was to attempt to locate the point P (Fig. 2) itself directly by a method of oscillation. This method requires a good deal of experience and practice and so is more

difficult than the first one, but all the same, it is more precise than (1). The difference of the reading (c) among themselves was of an order of 0.5 mm. The mean of ten such readings when compared with the mean of five pairs of readings, taken as described under (1) differed from each other. The order of difference was about 0.2 mm. But when the difference was found greater than this we placed more weight to the readings under (2) than those under (1). The following arbitrary curve (Fig. 2) which approximately represents the intensity of sound (as deduced from impression left on the memory) as the brass rod is moved in the vicinity of a maximum from one end to the other end situated symmetrically opposite to it, shows the accuracy of setting as well as the relative merits of methods (1) and (2) discussed above. The ends here do not mean exactly the limits between which the sound is audible; which in the case cited above would be about 5 or 6 mms. but by this we mean that we have to select two points such as A and A' of nearly equal intensity on either side of P and retain them in memory such that when the observations are repeated they may be located easily. These facts are illustrated from actual readings and may be considered as a typical example to clarify the principle of the method graphically, the readings below refer to the position of a maximum when the corresponding position of the second scratch on the brass rod, as read by the travelling microscope, was that given in table (3) below. The readings under (a) are the readings obtained while approaching the maximum and those under (b) taken while receding from it, and (c) are the readings obtained as described under method (2).

Table 3

Points.	Readings (a) in cms.	Points.	Readings (b) in cms.	Mean of (a) and (b) in cms.	Readings (c) in cms.
A	3.91	A'	4.10	4.005	4.02
B	3.95	B'	4.09	4.020	4.00
C	3.93	C'	4.06	3.995	3.98
D	3.91	D'	4.07	3.990	4.01
E	3.96	E'	4.03	3.995	4.02
F	3.95	F'	4.05	4.000	4.00
G	3.90	G'	4.02	3.960	3.99
					3.98
					3.99
					3.97
					3.98
					4.01
					4.02
					4.01
					4.00
				mean=3.999	mean=3.991

From the curve (Fig. 2) the superiority of method (2) to method (1) is obvious, for it is likely to judge all points in (a) group as lying between, say, G and C and all points in the (b) group as lying between, say, P and F, thus tending to shift the mean value, P, to some such point as E. The error might become serious if the difference between the readings in an individual pair, be of the order of about 4 or 5 mms.

### VIII

#### **Filling the Resonating Tube with Air completely Saturated with Water Vapour**

At first we tried this part of the experiment by simply blowing air through four wash bottles containing water and passing this air through the resonating tube for a few hours. The temperature of the fourth wash bottle, nearest to the gas inlet of the resonating tube was kept about five or six degrees higher than the actual temperature of the resonating tube, so that the final temperature of the saturated air, entering the resonating tube, was about the same as that of the air inside the resonating tube itself. In order to avoid the actual water particles entering the resonating tube, we placed wire meshes at several places in the path between the fourth wash bottle and the gas inlet. To ensure, that the air inside the resonating tube was at that temperature completely saturated with water vapour, some preliminary experiments were made. For this purpose, two special platinum thermometers, nearly identical to each other, were screwed, side by side, to the brass rod, R, and inserted in the resonating tube and the temperature of the air inside was thus read simultaneously by both the thermometers. Having done this, the bulb of one of the thermometers was covered with a piece of linen and a small glass tube, bent in nearly a semi-circular shape and closed at one end, to carry a little quantity of water, in order to keep the bulb of the thermometer wet, was fastened to the brass rod. The readings of the wet thermometer were found to be less than those of the dry thermometer by about one or two degrees, a fact which convincingly proved that the air thus saturated with water vapour by merely bubbling it through wash bottles containing water was not at all completely saturated. But at the same time, it was observed that the temperature of the wet thermometer was slowly rising, as time elapsed, so much so that after about an hour there was practically no difference in the readings of the wet and the dry thermometers. This showed us that the presence of water in the attached curved-tube and the wick of the thermometer, in a closed space like this, could slowly saturate the air completely in about less than an hour. It may be mentioned here that by quickly moving the brass rod, the temperature of the wet thermometer fell more than what it did when the brass rod was either stationary or very slowly

moved. In taking the wet and dry bulb readings the quick motion of the rod was avoided, so as to keep consistency with the usual convention of the dry and wet bulb hygrometry.<sup>10</sup> The importance of the necessity of a complete saturation of air in these experiments may be better realized by the actual experimental results given below.—

(a) The air was saturated by simply bubbling it through water and no water was introduced in the resonating tube. Under those conditions the velocity of sound in tube at  $37.5^{\circ}$  C. was found to be 355.54 m. per second.

(b) A little quantity of water was introduced at both the ends of the resonating tube and the readings were taken after about 15 minutes. The velocity of sound in this case was 356.28 m. per second.

(c) The third set of observations was taken after waiting for some time. The velocity in this case was 356.44 m. per second.

(d) The fourth set of observations was taken after a few minutes more. The velocity found was 356.43 m. per second.

(e) This set of observation was taken after waiting half an hour more. The velocity found was 356.38 m. per second.

These results show the process of saturation adopted in these experiments to be quite satisfactory. The process of saturating the air by mixing it thoroughly with steam and then filling the tube with the mixture was deliberately avoided for fear of the actual particles of water, in suspension in air, getting inside the resonating tube and creating other disturbances not desired.

## IX

### A Typical Set of Observations and Results

The temperature having been regulated and controlled, the resonating tube filled with the saturated air, as described, the internodal distances were located by hearing in the head phones and judging the points of maximum intensity of sound mostly by method (2) described under section VII. The observations were restricted only to the central portion of the tube about 100 cms. and a margin of about 35 cms. tube-length was left at each end of the tube. The arbitrarily chosen first maximum in table (4) was really the third node when counted from the mica membrane. The length of the tube was only 170 cms. and the internodal distance at about  $75^{\circ}$  C. was somewhat about 20 cms. and so only 5 maxima, at the most, could be located.

The pressure inside the resonating tube during the experiments was always kept equal to the atmospheric pressure which, however, was not constant, but the fluctuations in the atmospheric pressure, during a period of about three to four hours, were usually of an order of about two to four

mms. mercury column. Therefore to get a mean value of the pressure it was read on a Fortin's barometer at least three times during a run which, as noticed before, lasted three to four hours. The mean of the three readings of the barometer was then taken to indicate the true pressure inside the resonating tube for any particular experiment.

The observations of a typical experiment are set down in table 4 (p. 283).

From the same set of observations in table (4) the velocity of sound was calculated in three different ways as follows :—

Method (A): From table (4) and table (2), the distance between the 1st and the 3rd maximum

$$\begin{aligned}
 &= [9'180 - 3'504] + [30'17, \text{ which is the distance between the 2nd scratch} \\
 &\hspace{15em} \text{and the 5th scratch}] \\
 &= 5'676 + 30'170 = 35'846 \text{ cms.} \quad \dots \quad \dots \quad \dots \quad (1)
 \end{aligned}$$

In the same way the distance between the 2nd and the 4th maximum

$$\begin{aligned}
 &= [\text{the distance between the 3rd and the 7th scratch, which is } 40'150] - \\
 &\hspace{15em} [11'303 - 7'088] \\
 &= 35'925 \text{ cms.} \quad \dots \quad \dots \quad \dots \quad \dots \quad (2)
 \end{aligned}$$

Likewise the distance between the 3rd and the 5th maximum = [the distance between the 5th and the 9th scratch which is 40'150 cms]

$$\begin{aligned}
 &\quad \quad \quad - [9'174 - 5'016] \\
 &= 35'942 \text{ cms.} \quad \dots \quad \dots \quad \dots \quad \dots \quad (3)
 \end{aligned}$$

From these three values of  $\lambda$ , the mean wavelength is 35'907 cms.

The frequency of the source of sound = 994 cycles per sec.

$\therefore$  the velocity of sound in tube in saturated air at 37.5°C. is 356'92 m. per sec.

Method (B): From table (4) and table (2), the distance between the 1st and the 5th maximum as before =  $(5'016 - 3'504) + 70'200$  and hence  $\lambda = 35'856$  cms. and the velocity in saturated air in tube at 37.5°C. = 356'41 m. per sec.

Method (C): The calculations of the velocity of sound by this method may be better realised by a glance at table (5) given below, which, in addition to other facts, shows that the experimental values of the internodal distances are never exactly equal to each other. This may be at first thought of as due to the error of observation itself, but the consistency of results, all in thorough agreement with the statement made above tends to show that this may be due to some real complexities other than the error of observation. In calculating the velocity of sound by method (C) these facts are considered by giving equal weight to all the observations and taking the mean value thus obtained to represent the true value of the velocity of sound in tube. The values

Table 4

1st maximum 2nd scratch at			2nd maximum 3rd scratch at			3rd maximum 5th scratch at			4th maximum 7th scratch at			5th maximum 9th scratch at			Order of max.	Temp. deg. C	
Readings (a) cms.	Readings (b) cms.	Readings (c) cms.	Readings (a) cms.	Readings (b) cms.	Readings (c) cms.	Readings (a) cms.	Readings (b) cms.	Readings (c) cms.	Readings (a) cms.	Readings (b) cms.	Readings (c) cms.	Readings (a) cms.	Readings (b) cms.	Readings (c) cms.			
3.45	3.56	3.49	11.22	11.34	11.28	9.23	9.14	9.18	7.14	7.04	7.07	5.10	4.95	5.00	1st max.	37.5	
3.48	3.53	3.50	11.23	11.39	11.32	9.24	9.12	9.16	7.12	7.02	7.10	5.06	4.94	5.03			
3.43	3.54	3.52	11.26	11.38	11.30	9.22	9.13	9.18	7.15	7.05	7.09	5.07	4.96	5.01	2nd max.	37.5	
3.46	3.52	3.50	11.24	11.35	11.29	9.24	9.15	9.18	7.12	7.04	7.08	5.08	4.93	5.01			
3.43	3.53	3.49	11.25	11.36	11.30	9.25	9.12	9.17	7.14	7.03	7.10	5.05	4.95	5.02	3rd max.	37.5	
3.46	3.55	3.51	11.26	11.33	11.31	9.22	9.14	9.18	7.13	7.02	7.10	5.07	4.98	5.04			
3.42	3.57	3.52	11.24	11.35	11.32	9.24	9.13	9.18	7.13	7.04	7.09	5.07	4.97	5.03	4th max.	37.5	
3.44	3.54	3.49	11.23	11.36	11.30	9.23	9.11	9.16	7.14	7.03	7.07	5.06	4.98	5.04			
3.47	3.55	3.48	...	...	11.30	9.21	9.14	9.17	...	...	7.08	5.08	4.97	5.00	5th max.	37.5	
3.45	3.54	3.50	...	...	11.31	9.23	9.12	9.18	...	...	7.10	...	...	5.02			
3.45	3.57	3.52	...	...	...	...	...	...	...	...	...	...	...	5.03			
3.48	3.58	3.51	...	...	...	...	...	...	...	...	...	...	...	5.01			
...	...	3.49	...	...	...	...	...	...	...	...	...	...	...	5.00			
...	...	3.49	...	...	...	...	...	...	...	...	...	...	...	5.01			
...	...	3.51	...	...	...	...	...	...	...	...	...	...	...	5.02			
...	...	3.51	...	...	...	...	...	...	...	...	...	...	...	5.00			
...	...	3.52	...	...	...	...	...	...	...	...	...	...	...	5.02			
...	...	3.52	...	...	...	...	...	...	...	...	...	...	...	5.00			
...	...	3.51	...	...	...	...	...	...	...	...	...	...	...	5.00			
...	...	3.51	...	...	...	...	...	...	...	...	...	...	...	5.00			
mean of $\frac{a+b}{2}$	3.501	mean of $\frac{a+b}{2}$	11.299	mean of $\frac{a+b}{2}$	11.303	mean of $\frac{a+b}{2}$	9.180	mean of $\frac{a+b}{2}$	7.084	mean of $\frac{a+b}{2}$	7.088	mean of $\frac{a+b}{2}$	5.010	mean of $\frac{a+b}{2}$	5.016		
(c)	3.504	(c)	11.303	(c)	11.303	(c)	9.174	(c)	7.088	(c)	7.088	(c)	5.010	(c)	5.016		



given in table (5) are taken from the same set of observations contained in table (4)

Table 5

The distance between	Cms.	$\lambda/2$ in each case.	V in each case m/sec.	Percentage difference in V from the mean in each case.
1st and 2nd max. ...	17.829	17.829	354.44	-0.560
1st and 3rd " ...	35.796	17.898	355.81	-0.168
1st and 4th " ...	53.710	17.903	355.91	-0.140
1st and 5th " ...	71.712	17.928	356.41	-0.000
2nd and 3rd " ...	17.967	17.967	357.19	+0.224
2nd and 4th " ...	35.881	17.941	356.67	+0.056
2nd and 5th " ...	53.883	17.961	357.07	+0.168
3rd and 4th " ...	17.914	17.914	356.13	-0.084
3rd and 5th " ...	35.916	17.958	357.01	+0.168
4th and 5th " ...	18.002	18.002	357.88	+0.392
	mean	17.930	356.45	

Thus we see that the velocity of sound as calculated from the same set of observations but by different methods differ from each other, namely by methods

- (a)  $V = 356.92$  m/sec.
- (b)  $V = 356.41$  m/sec.
- (c)  $V = 356.45$  m/sec.

The final results of the velocity of sound given in table (6) and Fig. (3) have been calculated by method (C). The differences between the results calculated by (b) or (c) were negligible but those between the results calculated by (a) and (c) were of the order of 0.5 m/sec. The accuracy of the results is claimed to be of the order of about 1 in 1000 up to the temperature about 60°C. The accuracy\* of results above this temperature may be somewhat about 5 parts in 1000.

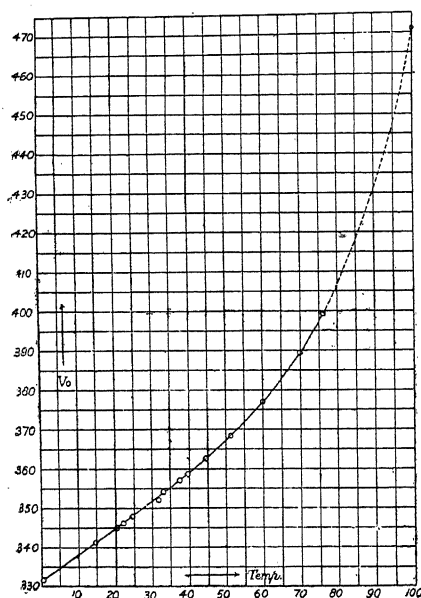


Fig. 3

\* The accuracy of results above 60°C. is a little less because at such temperatures the maxima were not sharp, probably due to the large absorption of sound by the saturated air, which, for instance, at 60°C. contains about 130 grams of moisture per cubic metre.

Table 6

Temperature.	V m/sec.	Tube* correction.	$V_0$ m/sec.
14.9°C	340.87	0.52	341.39
20.3°C	344.50	0.53	345.03
21.9°C	345.54	0.53	346.07
24.6°C	347.36	0.54	347.90
31.7°C	352.29	0.55	352.84
33.1°C	353.45	0.55	354.00
37.5°C	356.49	0.56	357.05
39.5°C	358.09	0.56	358.65
44.5°C	362.03	0.57	362.60
51.4°C	367.82	0.58	368.40
60.1°C	376.63	0.59	377.22
69.7°C	388.77	0.60	389.37
75.6	399.03	0.61	399.64
(100°C)†			(471.5)

## X

## The Tube Correction

Since the tube we have been using in our experiments has quite a smooth surface, we calculated the tube-correction by assuming the experimental value of Kirchhoff constant  $C$  to be correct for all practical purposes. Kaye and Sherratt,<sup>2</sup> after careful and extensive experimentation, of a high order of accuracy, have, concluded "that in general the Helmholtz-Kirchhoff equation is substantially valid for smooth tubes." Grüneisen and Merkel<sup>11</sup> who also agree with the form of the Helmholtz-Kirchhoff equation have obtained a value of  $C$  which is in accord with the value of  $C$  obtained by Kaye and Sherratt. These facts may be considered as sufficient to establish the validity of the much disputed expression

$$V = V_0 \left\{ 1 - \frac{C}{2r(\pi N)^{\frac{1}{2}}} \right\} \dots \dots \dots (3)$$

The mean values of  $C$  given by Kaye and Sherratt<sup>2</sup> for the case when dry air was used in glass tubes of smooth surface are 0.51 at 18°C. and 0.61 at 100°C.; the theoretical values of  $C$  for the same two temperatures being 0.56 and 0.69 respectively. The temperature range of our experiments extended from about 15°C. to about 80°C. Therefore in the present series of experiments there are also temperatures other than these two, namely, 18°C. and 100°C., thus rendering it necessary to know the values of  $C$  at these intermediate temperatures also. But the data for the experimental values of  $C$  at different temperatures ranging between 15°C. and 80°C. are at present not available; so that we thought it best to avoid this difficulty by an assumption

\* See Section X, table (8).

† W. G. Shilling, *Phil. Mag.*, Vol. 3, 293, 1927

Table 9

Diameter of glass tubes	Frequency	V m/Sec	V <sub>s</sub> m/Sec	Tube-correction	Temp.
*1.04 cms.	988.5	339.4	342.4	3.00	18°C
*2.89 cms.	988.5	341.4	342.4	1.00	18°C
6.08 cms.	994.0	344.29	344.83	0.53	20°C

## XI

The Calculation of  $\Gamma$ 

In the calculation of  $\Gamma$ , the ratio of the two specific heats for saturated

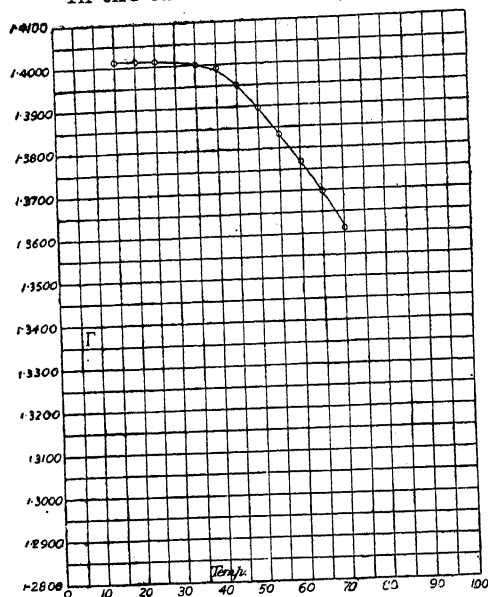


Fig. 5

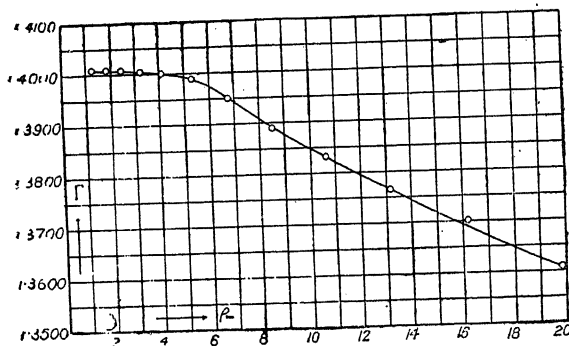


Fig. 6

The data for all such calculations and the results are given in table (10) below. Figs. (5) and (6) are the curves showing the variation of  $\Gamma$  with

air, the free-space velocities of sound at various temperatures were obtained from the smooth curve Fig. 3 drawn on a very large square paper. No correction for the imperfection of the saturated air deviating from the ideal gas law was made but simply the equation

$$V = \sqrt{\frac{P\Gamma}{\rho}}$$

was used.  $\rho_t$  the density of the saturated air at  $t^\circ\text{C}$ . was obtained by calculating  $\rho_0$ , the density of air at  $t^\circ\text{C}$ . and the partial pressure ( $P - p_{mt}$ ) and adding to this  $\rho_m$ , the density of the moisture present in the saturated air at  $t^\circ\text{C}$ . Here  $P$  is the total pressure of the mixture (i.e., saturated air) and  $p_{mt}$  is

the partial pressure of the moisture contained in the saturated air at  $t^\circ\text{C}$ . The densities of the moisture at various temperatures were calculated from Landolt's table, but more weight was given to the experimental values of the same obtained from a paper by J. H. Awbery (Proc. Phys. Soc., 44, 143, 1932).

\* Kaye and Sherratt, *Proc. Roy. Soc. A*, Vol. 141, p. 123, 1933.

Table 10

Temp.	$V_0$ m/sec in saturated air.	Total press. P mms.	Partial press. of moisture $p_m$ mms.	Partial press. of dry air $p_d$ mms.	Density of dry air at press. $p_d$ & $p_m$ .	Calculated density of moisture $p_m$ .	Experimental values of $p_m$ .	Density of saturated air $\rho = (\rho_m + \rho_d)$	$\gamma$ for saturated air.
15°C	341.55	742.1	12.78	729.3	$117.6 \times 10^{-5}$	$1.2 \times 10^{-5}$	...	$118.8 \times 10^{-5}$	1.4009
20°C	344.83	743.4	17.51	725.9	$115.0 \times 10^{-5}$	$1.8 \times 10^{-5}$	...	$116.8 \times 10^{-5}$	1.4008
25°C	348.15	744.3	23.69	720.6	$112.3 \times 10^{-5}$	$2.309 \times 10^{-5}$	$2.42 \times 10^{-5}$	$114.7 \times 10^{-5}$	1.4007
30°C	351.59	744.8	31.71	710.1	$108.9 \times 10^{-5}$	$3.041 \times 10^{-5}$	$3.17 \times 10^{-5}$	$112.1 \times 10^{-5}$	1.4005
35°C	355.30	743.3	42.02	701.3	$105.8 \times 10^{-5}$	$3.964 \times 10^{-5}$	$4.11 \times 10^{-5}$	$109.9 \times 10^{-5}$	1.4000
40°C	359.10	745.1	55.13	690.0	$102.4 \times 10^{-5}$	$5.118 \times 10^{-5}$	$5.28 \times 10^{-5}$	$107.7 \times 10^{-5}$	1.3989
45°C	363.05	745.6	71.6	674.0	$98.47 \times 10^{-5}$	$6.544 \times 10^{-5}$	$6.72 \times 10^{-5}$	$105.2 \times 10^{-5}$	1.3949
50°C	367.18	745.4	92.3	653.1	$93.93 \times 10^{-5}$	$8.299 \times 10^{-5}$	$8.47 \times 10^{-5}$	$102.4 \times 10^{-5}$	1.3893
55°C	371.70	745.0	117.8	627.2	$88.82 \times 10^{-5}$	$10.433 \times 10^{-5}$	$10.59 \times 10^{-5}$	$99.41 \times 10^{-5}$	1.3828
60°C	376.90	745.0	149.2	595.8	$83.12 \times 10^{-5}$	$13.011 \times 10^{-5}$	$13.13 \times 10^{-5}$	$96.25 \times 10^{-5}$	1.3765
65°C	382.80	745.0	187.3	557.8	$76.67 \times 10^{-5}$	$16.116 \times 10^{-5}$	$16.17 \times 10^{-5}$	$92.84 \times 10^{-5}$	1.3697
70°C	389.80	745.0	233.5	511.5	$69.27 \times 10^{-5}$	...	$19.76 \times 10^{-5}$	$89.02 \times 10^{-5}$	1.3618
75°C	398.60	745.0	299.1	445.9	$59.51 \times 10^{-5}$	...	$23.99 \times 10^{-5}$	$83.49 \times 10^{-5}$	
80°C	408.00	745.0	355.1	389.9	$51.28 \times 10^{-5}$	...	$28.92 \times 10^{-5}$	$79.7 \times 10^{-5}$	

the temperature and with the moisture content of the saturated air at that temperature respectively.

## XII

### The Calculations of $\gamma_m$

In order to calculate the values of  $\gamma_m$  for the moisture contained in the saturated air at different temperatures the values of  $\gamma_D$  for dry air at these temperatures were obtained by taking the most probable experimental values of the velocity of sound in dry air at the same temperature. But the selection of these values was restricted to only such tube experiments in which due care was taken to make the apparatus really air-tight. The values of  $\gamma_D$  for dry air were then calculated without applying any correction for the little deviation of dry air from the ideal gas law. The values of the velocity of sound in dry air taken from other workers together with the values of  $\gamma_D$  for dry air determined by other experimental methods are also set down in table (11) below.

Table 11

Temperature.	$V_0$ m/sec.	$\rho_D$ at 760 mms. pressure	$\gamma_D$ calculated.	Reference.
0°C	331.5	$129.3 \times 10^{-5}$	1.4023	Tube method, Kaye <sup>2</sup> and Sherratt.
14°C	339.8	$123.0 \times 10^{-5}$	1.4016	Tube method.
18°C	342.3	$121.4 \times 10^{-5}$	1.4027	Kaye <sup>2</sup> and Sherratt.
100°C	387.3	$94.64 \times 10^{-5}$	1.4017	" "
0°C	331.44	$129.28 \times 10^{-5}$	1.4017	In free air, Hebb.
Temperature.	$\gamma_D$ corrected.	..	$\gamma_D$ not corrected.	...
0°C	1.4025	..	1.4012	Lummer and Pringsheim.
0°C	1.4003	...	1.3992	Moody.
0°C	1.4034	...	1.4021	Partington.
0°C	1.4029	...	1.4016	Shields.
0°C	1.4026	..	1.4012	Hebb (1904)
0°C	1.4031	...	1.4017	Hebb (1919)

Having done this, we have calculated the values of  $\gamma_m$ , the ratio, of the two specific heats, for the moisture contained in saturated air at various temperatures by using Einstein's theoretical expression\*:

$$\frac{P}{\Gamma-1} = \frac{p_D}{\gamma_D-1} + \frac{p_m}{\gamma_m-1} \quad (5)$$

The data for all such calculations and the results are given in table (12) below:

Table 12

Temp °C.	Vap. pressure mms.	$p_m$	$\Gamma$	$\gamma_D$	$\gamma_m$
15°C	12.78	$1.2 \times 10^{-5}$	1.4009	1.4021	1.3421
20°C	17.51	$1.8 \times 10^{-5}$	1.4008	1.4020	1.3568
25°C	23.69	$2.42 \times 10^{-5}$	1.4007	1.4019	1.3672
30°C	31.71	$3.17 \times 10^{-5}$	1.4005	1.4018	1.3736
35°C	42.02	$4.11 \times 10^{-5}$	1.4000	1.4018	1.3723
40°C	55.13	$5.28 \times 10^{-5}$	1.3989	1.4017	1.3671
45°C	71.60	$6.72 \times 10^{-5}$	1.3949	1.4017	1.3406
50°C	92.30	$8.47 \times 10^{-5}$	1.3893	1.4016	1.3199
55°C	117.80	$10.59 \times 10^{-5}$	1.3828	1.4016	1.3054
60°C	149.2	$13.13 \times 10^{-5}$	1.3765	1.4015	1.3015
65°C	187.3	$16.17 \times 10^{-5}$	1.3697	1.4014	1.2993
70°C	233.5	$19.76 \times 10^{-5}$	1.3618	1.4013	1.2976
75°C	299.1	$23.99 \times 10^{-5}$	...	...	...
80°C	355.1	$28.92 \times 10^{-5}$	...	...	...
85°C	433.5	$34.67 \times 10^{-5}$	...	...	...
90°C	525.8	$41.28 \times 10^{-5}$	...	...	...

\* It may however be noted that the expression

$$\frac{1}{\Gamma-1} = \frac{\rho-\rho_D}{\rho_m-\rho_D} \cdot \frac{1}{\gamma_m-1} + \frac{\rho_m-\rho}{\rho_m-\rho_D} \cdot \frac{1}{\gamma_D-1}$$

due to Richarz (Ann. Phys. 1906) is the same as the expression (5).

Table 13

Temperature	$V_H$ , the free-space velocity in saturated air	$V_0$ , the free-space velocity in dry air	$V_H - V_0$ m/sec.
0°C	331.60	331.50	0.1
10°C	...	337.6	
15°C	341.55	340.40	1.15
20°C	344.8	343.5	1.32
25°C	348.1	346.35	1.75
30°C	351.5	349.3	2.2
35°C	355.3	352.1	3.2
40°C	359.1	354.95	4.15
45°C	363.05	357.8	5.3
50°C	367.2	360.7	6.5
55°C	371.7	363.4	8.3
60°C	376.9	366.2	10.7
65°C	382.8	368.9	13.9
70°C	389.8	371.6	18.2
75°C	398.6	374.3	24.3
80°C	408	377	about 31
(100°C)	(471.5)*	(387.3)†	(84.2)

## ACKNOWLEDGMENTS

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CHEMICAL EXAMINATION OF THE SEEDS OF  
*ABRUS PRECATORIUS*, LINN. PART III.  
THE CONSTITUTION OF ABRINE

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In part I of this series of investigations<sup>1</sup> the seeds of the important medicinal plant *Abrus precatorius*, Linn. of the natural order Leguminosae, or Jequirity as it is known in English and Rati in Hindustani, were chemically examined. A colourless crystalline nitrogenous compound in the form of slender needles was isolated and named as 'abrine' which is most probably the active principle of the seeds. The pharmacological examination of the substance is in progress in the King George's Medical College, Lucknow. In the present paper it is intended to elucidate the constitution of abrine as far as it has been possible by the preparation of some of its derivatives.

Abrine is present in the kernels of the seeds of *Abrus precatorius*. In pure form it has no smell and is tasteless. It crystallizes in minute silky white needles from alcohol. In water it is sparingly soluble in the cold and crystallizes well from a boiling solution. If it is allowed to crystallize very slowly extending over several days, abrine is obtained in the form of stout, long crystals in the form of stars. Careful crystallization may increase the length of the crystals from two to three centimetres. It has a high melting point at 295°. When heated over flame in a dry test tube abrine melts, becomes brown with decomposition and gives out white fumes which have a very disagreeable odour. The fumes condense in the cooler parts of the tube forming a yellowish white liquid. When heated with zinc dust it gives out the same smell as above along with that of ammonia.

Abrine forms mono-hydrochloric and mono-nitric acid salts with the corresponding acids. It thus behaves as a mono-basic compound. Abrine does not produce any coloration with neutral ferric chloride solution but forms a mono-acetyl and mono-phenylurethane, proving thereby the presence of an alcoholic hydroxy group. The formation of a dibromo derivative with bromine proves the presence of a double bond in abrine. This is also borne out by volumetric estimation of unsaturation in abrine. Abrine forms a mono-nitroso derivative, thus showing the presence of a



secondary amino group. The facts that abrine does not respond to the *ninhydrin-reaction* and its aqueous solution is not coagulated by tannic acid show that it has no amino-acidic group in it. Abrine forms a mono-nitro derivative and is readily oxidised by solution of potassium permanganate.

#### EXPERIMENTAL

It has already been shown<sup>1</sup> that the most suitable solvent for the extraction of abrine from the seeds of *Abrus precatorius* is alcohol, which takes out the substance in very small quantities. Solubility of abrine in water could not be used with advantage for its extraction as the powdered kernel of the seeds swells to a considerable extent in presence of water and working with such gelatinous mass becomes very difficult. The swelling is due to the presence of large quantities of albuminous and mucilagenous substances present in the kernels of the seeds.

For extraction of abrine, the hard yellow kernels were ground to a fine powder in a hand mill and was extracted in a big Soxhlet's extraction apparatus, with petroleum ether. When the oil was completely removed by the solvent the powder was freed from petroleum ether and exhaustively extracted with rectified spirit. With the progress of extractions the yellow colour of the powder became lighter and finally became white. The total alcoholic extract was concentrated to a small volume under reduced pressure. The thick brown syrup, which had a disagreeable odour, contained fine needle-shaped crystalline suspension. It was allowed to stand for about ten days when the quantity of the crystalline deposit increased. The product was separated and washed with benzene to remove oily contamination. It was next washed with small quantities of cold water which readily removed the sticky portions. The residue on crystallization from boiling water was obtained as fine silky white needles. As has previously been recorded, it has a molecular formula  $C_{12}H_{14}O_2N_2$  and has been named "abrine". The general reactions of abrine have also been recorded. Its solution is not coagulated by tannic acid solution and is non-respondent to the *ninhydrin-reaction* of amino-acids.

#### Abrine hydrochloride, $C_{12}H_{14}O_2N_2 \cdot HCl$

To 0.5 g. of abrine in a dry dish was added one c.c. of pure concentrated hydrochloric acid. Abrine immediately dissolved but very soon the whole of it was precipitated as white needle shaped crystals. The liquid contamination of the crystals was soaked out by means of filter papers. The product was dried in a vacuum desiccator over calcium oxide. It was then washed several times with dry ether to free it from traces of hydrochloric acid. It melted at  $221.5^\circ$  sharp. When slowly allowed to crystallize from fairly strong

hydrochloric acid, abrine hydrochloride separates in the form of long stout needles in starry clusters.

[Found : Cl=14.25 %;  $C_{12}H_{15}O_2N_2$  Cl, requires Cl=13.94%.]

Abrine hydrochloride dissolves in water but soon after a flocculent white precipitate separates. On analysis the precipitate has been found to be pure abrine and the filtrate contains hydrochloric acid. The combination of abrine with hydrochloric acid is therefore fairly weak and their detachment is effected in presence of water.

**Abrine nitrate,  $C_{12}H_{14}O_2N_2 \cdot HNO_3$**

0.5 g. of abrine was put in a small clean beaker and very dilute nitric acid was added slowly till the whole of abrine just dissolved. Excess of acid was avoided. The solution was kept for spontaneous evaporation. After few days long white needles of abrine nitrate separated. The crystals were dried within filter papers and finally in a vacuum desiccator over calcium oxide. It was finally washed with dry ether and on drying melted at  $143^\circ$  with decomposition. Abrine nitrate is very soluble in water and the combination of the salt is very stable in presence of the solvent.

[Found : N=15.42%;  $C_{12}H_{15}O_5N_3$ , requires N=14.94%.]

**Abrine picrate,  $C_{12}H_{14}O_2N_2 \cdot C_6H_3O_7N_3$**

1 g. of picric acid was dissolved in 30 c.c. of alcohol (98 %) and 0.5 g. of abrine was added. On slight warming, abrine dissolved and the colour of the solution slowly darkened and finally became orange-red. It was then heated to boil and allowed to stand overnight. Next morning orange-yellow crystalline plates in clusters were formed. The mother liquor was decanted off and the crystals were washed free from picric acid with dilute alcohol. The picrate weighed 0.9 g. and melted at  $194^\circ$  with decomposition. It was quite stable in presence of water in which it was very little soluble, forming faint yellow solution.

[Found : N=16.06 %; 15.92%;  $C_{18}H_{17}O_9N_5$ , requires N=15.66%].

The picrate was also prepared in glacial acetic acid from which two different types of crystals were obtained—(1) orange-red needles in form of stars, and (2) very closely packed soft yellow needles. Both melted at  $194^\circ$  with decomposition and the nitrogen content was 15.96 per cent and 15.89 per cent respectively. The orange-red variety changed colour and became perfectly yellow at  $120^\circ$ . Thus all the three varieties were mono-picric acid salts of abrine having only different crystalline modifications.

**Dibromo abrine,  $C_{12}H_{14}O_2N_2Br_2$**

0.5 g. of abrine was put in a dry flask and alcoholic solution of bromine was added in the cold. The colour of bromine was discharged and abrine

dissolved forming a light pink solution. Excess of bromine solution was added and was left for spontaneous evaporation at room temperature. After few days soft yellow plates settled at the bottom and the mother liquor remained brown. Addition of water did not separate more of the bromo derivative. Some acetone was next added when the solid deposit became colourless and the brown mother liquor was decanted off. It was thus freed from bromine by two more washings with acetone. The product on drying was obtained as whitish micro-crystalline powder. It slowly started turning dark from  $220^{\circ}$  and melted between  $241-42^{\circ}$ , with decomposition.

[Found Br. = 42.7 %;  $C_{12}H_{14}O_2N_2Br_2$ , requires Br = 42.3%.]

The volumetric estimation of unsaturation in abrine was carried out as follows:—0.2974 g. of abrine was dissolved in 10 c.c. of carbon tetrachloride in a stoppered 250 c.c. measuring flask and N/3-bromine (20 c.c.) in the same solvent added and the mixture allowed to stand in a dark place for 24 hours. The mixture was then cooled in ice and water (25 c.c.) quickly added and well shaken and then 10 per cent potassium iodide solution (25 c.c.) with water (75 c.c.) introduced and the whole thoroughly agitated. The iodine thus liberated was titrated against N/10 sodium thiosulphate. After titration, 2 per cent potassium iodate (5 c.c.) was added and the titration repeated. Twice this value was deducted from the above titration value and the equivalents of bromine atoms taken up by abrine molecule calculated, which came to 1.97. This means one double bond in abrine.

#### Nitro-abrine, $C_{12}H_{13}O_2N_2.NO_2$

1 g. of abrine and 20 c.c. of nitric acid (d. 1.2) was put in a nitration flask. Abrine dissolved with evolution of heat and the colour of the solution became orange-red. In about an hour the whole of abrine was dissolved. It was refluxed for about 5 hours over water bath. On cooling a pasty brown mass settled at the bottom. The mother liquor on dilution with water deposited a flocculent yellow mass. The precipitate was washed, dried and crystallized from dilute alcohol. When heated, it shows no sign of melting, darkens at about  $185^{\circ}$  and finally decomposes at about  $220^{\circ}$ , being then a carbonaceous powder. The product was proved to be a nitro compound by silver deposit test (zinc, alcoholic silver nitrate and substance).

[Found N = 16.3 %;  $C_{12}H_{13}O_4N_3$ , requires N = 16.0 %.]

#### Nitroso-abrine, $C_{12}H_{13}O_2N_2.NO$

To a cooled solution of 0.5 g. of abrine in 20 c.c. of 10 per cent acetic acid was added a well cooled solution of 0.25 g. of sodium nitrite in 10 c.c. of water.

An yellow coloured precipitate was formed which was filtered. It was washed with 5 per cent acetic acid and then with water. It was dried in a vacuum desiccator when it melted at  $121^{\circ}$ .

[Found N=17.2 % ;  $C_{12} H_{13} O_3 N_3$ , requires N=17.0 %.]

**Acetyl abrine,  $C_{12} H_{13} ON_2 \cdot O \cdot COCH_3$**

1 g. of abrine was refluxed with 10 c.c. of acetic anhydride and fused sodium acetate for about an hour. It was cooled. On addition of cold water a brownish yellow paste separated, which solidified becoming brittle on long standing. It was twice crystallized from dilute alcohol and animal charcoal, when it was obtained as a white microcrystalline power. It melted at  $286-287^{\circ}$  with decomposition.

[Found N=10.9 % ;  $C_{14} H_{16} O_3 N_2$ , requires N=10.8 %]

**Abrine-phenylurethane,  $C_{12} H_{13} ON_2 \cdot O \cdot CO \cdot NHC_6H_5$**

0.7 g. of abrine and 10 c.c. of phenylisocyanate was kept in a dry flask and refluxed over water-bath, carefully avoiding the entry of water vapour into the flask. Abrine dissolved slowly and the colour of the mother liquor became yellow. On allowing the product to stand at ordinary temperature for some time the phenylurethane product crystallized out which was filtered off and washed with benzene till the smell of phenylisocyanate had completely disappeared. It was recrystallized from alcohol, when it was obtained as white needles. It melted at  $271^{\circ}$ .

[Found N=12.8 % ;  $C_{19} H_{19} O_3 N_3$ , requires N=12.5 %.]

The author wishes to express his indebtedness to the "Lady Tata Memorial Trust" of Bombay for a scholarship which enabled him to take part in the investigation.

*Reference*

1. Ghatak and Kaul, *J. Indian Chem. Soc.*, 9, 383, 1932.



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